Volume 26, Number 5, April, 1954

The Yale Journal of Biology & Medicine

345 **EFFECT OF CORTISONE ON 17-KETOSTEROID EXCRETION** *Joseph E. Sokal, Philip K. Bondy, Pasquale J. Costa, Clyde L. Deming, and B. Marvin Harvard, Jr.*

352 **VIRUS ENZYME AND MUCOPROTEIN** *Alfred Gottschalk*

365 **PLASMINOGEN** *Daniel L. Kline*

372 **MASSIVE INTRAVENOUS INFUSIONS** *William Harrison and Averill A. Liebow*

385 **COMPLEMENT FIXATION TESTS IN POLIOMYELITIS** *Francis L. Black and Joseph L. Melnick*

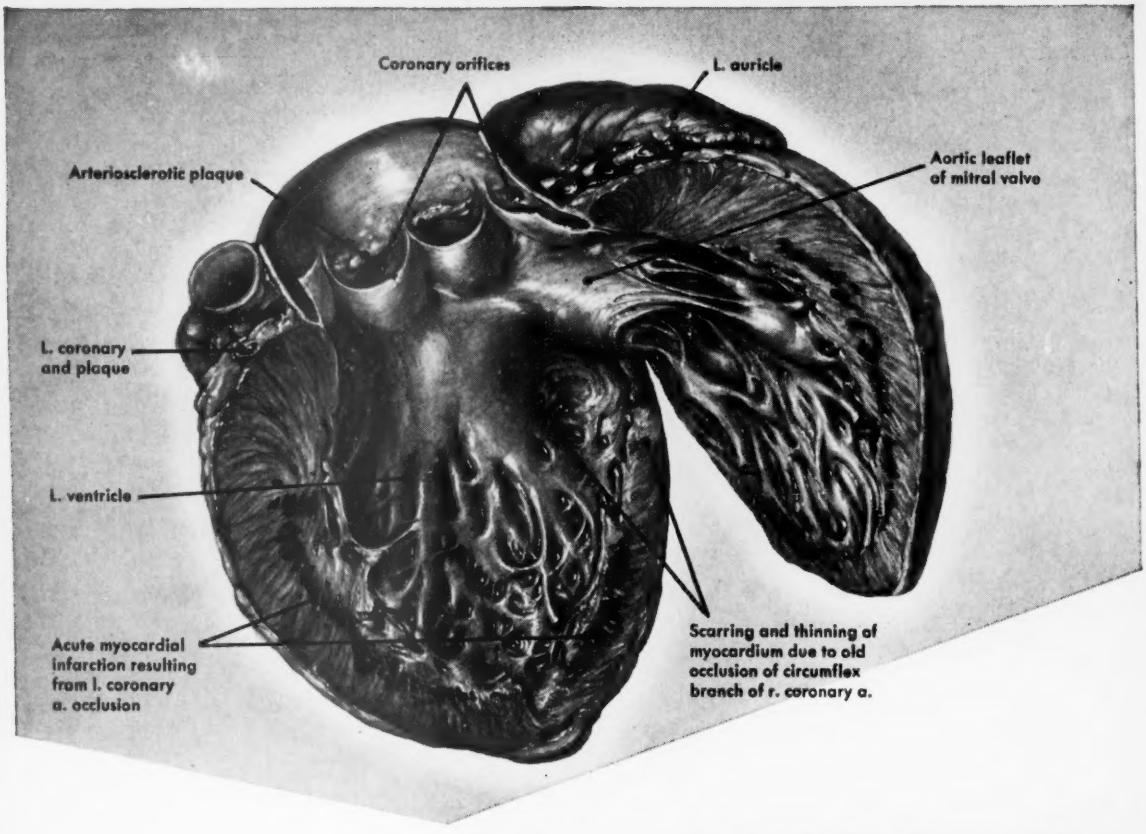
394 **AUROTHIOGLUCOSE OBESITY** *Robert H. Drachman and Jay Tepperman*

410 **WILLIAM HARVEY'S USE OF QUANTITATIVE METHOD** *Frederick G. Kilgour*

422 **HENRY BUNTING 1911-1954** *Thomas C. Mendenhall*

424 **EVENTS**

434 **BOOK REVIEWS**


Coronary orifices
L. auricle
Arteriosclerotic plaque
Aortic leaflet of mitral valve
L. coronary and plaque
L. ventricle
Acute myocardial infarction resulting from l. coronary a. occlusion
Scarring and thinning of myocardium due to old occlusion of circumflex branch of r. coronary a.

Myocardial Infarction with Severe Shock:

Reduction of Mortality

"... in correcting the state of shock we have enabled certain patients to recover from the initial episode, which otherwise gave every indication of culminating in death."[1]

Levophed infusion causes a striking rise in oxygen tension in areas of myocardial ischemia produced by coronary obstruction.[2] "It is therefore reasonable to suppose that the restoration of arterial pressure to a level consistent with an adequate coronary flow must limit the size of the infarct, save healthy myocardium, shorten the period of shock, and lessen the likelihood of congestive failure at a later date."[3]

"Severe cardiogenic shock demands therapy...the most efficacious method of treatment now available is use of vasopressor drugs."[4] "Current usage favors nor-epinephrine."[5]

References:

1. Kurland, G. S., and Malach, Monte: *New England Jour. Med.*, 247:383, Sept. 11, 1952.
2. Sayen, J. J., et al.: *Jour. Clin. Investigation*, 31:658, June, 1952.
3. Gilchrist, A. R.: *Brit. Med. Jour.*, 2:351, Aug. 16, 1952.
4. Miller, A. J., et al.: *J.A.M.A.*, 152:1198, July 25, 1953.
5. Levine, H. D., and Levine, S. A.: *Med. Clin. North America*, 37:955, July, 1953.

WINTHROP-STEARNS INC.
New York 18, N. Y. • Windsor, Ont.

WINTHROP

Levophed®

BITARTRATE

Brand of Levarterenol bitartrate *(nor-epinephrine)*

THE MOST POWERFUL PRESSOR ANTIDOTE FOR

Shock

Supplied: Levophed bitartrate solution 0.2 per cent (equivalent to 0.1 per cent base), ampuls of 4 cc., boxes of 10. (Each 4 cc. to be administered in 1000 cc. of 5 per cent dextrose.)

Levophed, trademark reg. U.S. Pat. Off.

CONTENTS

345 **THE EFFECT OF CORTISONE ON 17-KETOSTEROID EXCRETION OF PATIENTS WITH CARCINOMA OF THE PROSTATE** *Joseph E. Sokal, Philip K. Bondy, Pasquale J. Costa, Clyde L. Deming, and B. Marvin Harvard, Jr.*

352 **THE INFLUENZA VIRUS ENZYME AND ITS MUCOPROTEIN SUBSTRATE** *Alfred Gottschalk*

365 **STUDIES ON THE PURIFICATION AND ACTIVATION OF PLASMINOGEN (PROFIBRINOLYSIN)** *Daniel L. Kline*

372 **THE EFFECTS OF MASSIVE INTRAVENOUS INFUSIONS WITH SPECIAL REFERENCE TO PULMONARY CONGESTION AND EDEMA** *William Harrison and Averill A. Liebow*

385 **THE SPECIFICITY OF THE COMPLEMENT FIXATION TEST IN POLIOMYELITIS** *Francis L. Black and Joseph L. Melnick*

394 **AUROTHIOGLUCOSE OBESITY IN THE MOUSE** *Robert H. Drachman and Jay Tepperman*

410 **WILLIAM HARVEY'S USE OF THE QUANTITATIVE METHOD** *Frederick G. Kilgour*

422 **HENRY BUNTING 1911-1954** *Thomas C. Mendenhall*

424 **EVENTS**

434 **BOOK REVIEWS**

THE YALE JOURNAL
OF BIOLOGY AND MEDICINE

Founded in 1928.

George H. Smith; *Editor-in-Chief, 1928-49; Editor-in-Chief Emeritus, 1949-52.*

CONSULTING EDITORS

Paul B. Beeson; Joseph S. Fruton; Gustaf E. Lindskog; John S. Nicholas; Milton J. E. Senn; Herbert Thoms; Milton C. Winternitz.

BOARD OF EDITORS

Peter Blos, Jr.; Edgar J. Boell; Padraic Burns; David W. Calhoun; Joseph C. Cerny; Edward D. Coppola; Milton Corn; Gary A. Fry; Norman H. Giles, Jr.; Jack Peter Green; Robert R. L. Guillard; Nicholas A. Halasz; Marie-Louise Johnson; Frederick G. Kilgour, *Managing Editor;* Gerald Klatskin; Peter H. Klopfer; James W. Lewis, *Advertising Manager;* Averill A. Liebow; Guy M. McKhann; Jacques M. Quen; John Keith Rose; Max Taffel; Elizabeth H. Thomson, *Assistant Editor.*

Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in September, November, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $6.00 in the United States, $7.50 in other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

Entered as second-class matter January 11, 1929, at the post office at New Haven, Connecticut, under the Act of March 3, 1879.

PRINTED BY THE VAN DYCK PRINTING CO., NEW HAVEN, CONN.

Upjohn

tablet tonic:

Cebēfortis Tablets

TRADEMARK, REG. U. S. PAT. OFF.

...and hematonic:

Cebētinic Tablets

TRADEMARK, REG. U. S. PAT. OFF.

Each Cebēfortis Tablet contains:

Ascorbic Acid	150.0 mg.
Thiamine Hydrochloride	5.0 mg.
Riboflavin	5.0 mg.
Pyridoxine Hydrochloride	1.0 mg.
Calcium Pantothenate	10.0 mg.
Nicotinamide	50.0 mg.
Folic Acid	0.75 mg.
Vitamin B_{12} Activity	2.0 mcg.

Bottles of 100 and 500 tablets

Each Cebētinic Tablet contains:

Ascorbic Acid	25.0 mg.
Thiamine Hydrochloride	2.0 mg.
Riboflavin	2.0 mg.
Pyridoxine Hydrochloride	0.5 mg.
Nicotinamide	10.0 mg.
Ferrous Gluconate	5.0 grs.
Folic Acid	0.67 mg.
Vitamin B_{12} Activity	5.0 mcg.

Bottles of 60 and 500 tablets

The Upjohn Company, Kalamazoo, Mich.

BASIC to the practice of medicine

Tetra

BASIC among broad-spectrum antibiotics

basic chemically

representing a nucleus of modern broad-spectrum activity

basic clinically

true broad-spectrum action against pneumococci, streptococci, staphylococci and other gram-positive and gram-negative pathogens

unexcelled tolerance

outstanding stability and solubility

quickly reaches and maintains high therapeutic levels

may often be used successfully in patients in whom resistance or sensitivity to other forms of antibiotic therapy has developed

AIH *(afebrile in hours)*

*"Symptoms, including fever, largely cleared up within 24 to 48 hours."**

cyn

brand of **tetracycline**

TETRACYN TABLETS *(sugar coated)*
250 mg., 100 mg., 50 mg.

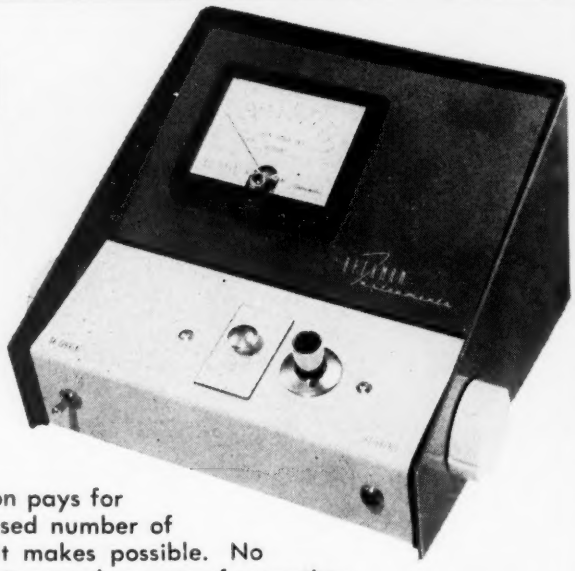
TETRACYN INTRAVENOUS
Vials of 250 mg. and 500 mg.

TETRACYN ORAL SUSPENSION
(amphoteric) (chocolate flavored)
Bottles of 1.5 Gm.

*English, A. R., et al.:
Antibiotics Annual (1953-1954), New York,
Medical Encyclopedia, Inc., 1953, p. 70.

536 Lake Shore Drive, Chicago 11, Illinois

THE BECKMAN CLINICAL COLORIMETER



- **Direct-reading Meter**
- **Pinpoint Beam**
- **Easy to Use**

This new colorimeter soon pays for itself through the increased number of readings per operator it makes possible. No other colorimeter equals its speed or ease of operation. A modern maintenance-free instrument with superior optical system. Unaffected by vibrations. Porcelain case is corrosion-proof.

For more detailed information call or write

MACALASTER BICKNELL COMPANY

183 Henry Street — New Haven, Connecticut — MAin 4-4191-2-3

Journal of the History of Medicine and Allied Sciences

Publishes Original Articles in All Spheres of the History of Medicine and of the Disciplines Ancillary to It

Contents of April issue

Thomas Dover 1662-1742. Physician, Privateering Captain, and Inventor of Dover's Powder, *D. N. Phear*

Ancient Alchemy and Abstract Art, *George Sarton*

Coffeehouse Cures, *John J. Keevil*

Andreas Vesalius, Count Palatine, *Charles Donald O'Malley*

De Graaf's "Tractatus de Clysteribus," *William Brockank* and *O. R. Corbett*

Published quarterly

Yearly subscriptions $7.50 Student subscriptions $5.00

DEPARTMENT OF THE HISTORY OF MEDICINE

333 Cedar Street, New Haven 11, Connecticut

Kodak reports to laboratories on:

making pH indicator solutions easier to use... some things you can do with oxidized cellulose... how 3 x 5-inch file cards turn into a library

Indicator solutions

In the more orderly laboratories, it hangs on the wall; in others, you have to rummage for it under somebody's desk blotter. Ubiquitous on the scientific scene it is, though—the famous Eastman pH Indicator Chart with its array of bars that tell at a glance which of some 50-odd Eastman Organic Chemicals changes from what color to what other color over what pH range.

We now report that nearly all the Eastman Indicators are now available as solutions in 500-cc bottles, with solvent, concentration, and pH range stated on the label. We continue to sell the indicators undissolved also.

Direct your inquiries and orders for Eastman pH Indicator Solutions to Distillation Products Industries, Eastman Organic Chemicals Department, Rochester 3, N. Y. (Division of Eastman Kodak Company). Write to the same address if the Eastman Indicator Chart is still not quite ubiquitous enough for your convenience. (It's free.)

Slightly degenerated cotton

Instead of oxidizing cellulose as man has done from time immemorial to take the chill out of his bones, cook his dinner, and dispel his gloom, we have a trick of oxidizing it only to a 10% to 22% carboxyl level. This we do by treatment with nitrogen dioxide. The product, called, surprisingly enough, "oxidized cellulose," looks like this:

It has the appearance and even some of the strength of ordinary cotton, along with some rather extraordinary properties. The most recent of these to come to light is its use in the diagnosis of congenital hyperplasia of the adrenal gland in children. This comes about from the remarkable affinity of oxidized cellulose for ACTH, which it absorbs quantitatively. ACTH is not detectable in the blood of normal children but does appear where this affliction exists. After cortisone treatment, no ACTH is detectable. One advantage of oxidized cellulose in ACTH assay is that the concentrates it yields are not toxic to the assay animal.

This is not the debut of the stuff as a tool in the healing art. Its first flush of prominence a few years ago was based on the surgically useful fact that it has the physical attributes of cotton gauze, yet is wholly absorbed in the blood stream after fulfilling its mechanical function.

Aside from its medical use, oxidized cellulose is an ion exchange medium, plucking cations out of aqueous solution to form metallic salts of cellulose. Plain cotton as far as the eye can see and the finger can feel, it bewitchedly vanishes into solution when popped into a 2% sodium hydroxide solution. When kept dry and cold, however, it survives indefinitely without change.

Trimming the cellulose chains with 10 to 22 percent of carboxyls drives the price of cotton up to $62.25 per pound, FOB Kingsport, Tenn. If you are interested in concentrating some ACTH, this may be a bargain for you right now, and if you can develop some decent volume of demand for it, we'll probably drop the price. For the cold facts, write Eastman Chemical Products, Inc., Chemical Division, Kingsport, Tenn. (Subsidiary of Eastman Kodak Company).

Microprint

It's the brutal truth that a man or woman is covering a narrow field indeed if he or she can honestly claim to be abreast of all that's set down on paper about it. A remedy—microprint cards—has been proposed by which a library card catalog can replace the library itself. Almost a decade of development has demonstrated its merit. Since it is based on photography, the time has come to state our position:

Before 1954 ends, you will be able to walk into dealers' establishments throughout the United States and be shown the *Kodagraph Microprint Reader*. This is an instrument, weighing less than a standard typewriter, for reading microprint cards with complete comfort. Microprint cards, usually the standard 3″ x 5″, look like the familiar library catalog card, carrying classification data, perhaps a brief abstract, etc., but, instead of having then to locate the item cataloged if it seems pertinent, it's right there on the back of the card in microprint—as many as 60 pages of the actual text.

"Complete comfort" is very important. Without it there would be no spreading of microprint readers from large libraries to smaller ones and on down to the individual user's office, desk, and even home. With large numbers of users to share the cost, microprint card literature will become even vastly more extensive and intensive than it has already grown. Economic barriers to the development of automatic subject-searching equipment will fall. New microprint publishing ventures will flourish—some for profit, some for the promotion of scholarship in fields too sparsely inhabited to support the cost of conventional publication.

Our part is to work closely with everybody, supplying technical hints, *Kodagraph Microprint Readers*, *Kodagraph Micro-File Film*, *Kodagraph Microprint Paper*, and equipment to turn out microprint cards by the piece or peck.

If the possibilities of microprint interest you, we'd appreciate your dropping a note to Eastman Kodak Company, Industrial Photographic Division, Rochester 4, N. Y., to let us know the nature of that interest.

Price quoted is subject to change without notice.

Kodak

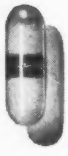
TRADE-MARK

This is one of a series of reports on the many products and services with which the Eastman Kodak Company and its divisions are... *serving laboratories everywhere*

truly one of the world's outstanding therapeutic agents

Chloromycetin®

(Chloramphenicol, Parke-Davis)



The widespread and discerning use of a medicinal product by physicians, in hospitals and in private homes—by day and by night, and in the treatment of patients of all ages—constitutes, we believe, the true proving ground which singles out and gives recognition to that product's place in the practice of medicine.

More than 11,000,000 patients have been treated with CHLOROMYCETIN. Today its vast "proving ground" reaches out and extends into practically every country of the civilized world.

PARKE, DAVIS & COMPANY DETROIT 32, MICHIGAN

JOSEPH E. SOKAL
PHILIP K. BONDY
PASQUALE J. COSTA
CLYDE L. DEMING
B. MARVIN HARVARD, JR.

Departments of Internal Medicine, Pharmacology, and Urology, Yale University School of Medicine

THE EFFECT OF CORTISONE ON 17-KETOSTEROID EXCRETION OF PATIENTS WITH CARCINOMA OF THE PROSTATE*

In 1952 cortisone therapy was initiated in two patients with far advanced prostatic carcinoma.[2] As part of the study of those patients, determinations were made of urinary 17-ketosteroid excretion before and during therapy with cortisone. Rather large increases in excretion of 17-ketosteroids were noted during the administration of high doses of cortisone in both patients. This unexpected finding led to an investigation of the effect of cortisone on 17-ketosteroid excretion, the preliminary results of which are now being reported.

The following somewhat arbitrary procedure was adopted for the initial phase of this study. Three 24-hour urine samples were collected for base-line 17-ketosteroid determination. Cortisone acetate was then administered orally for three days in four doses of 75 mg. each, a total of 300 mg. per day; three 24-hour urine specimens were collected during this "cortisone period." Whenever possible, the experiment was completed during six consecutive days, during which no major change in the patient's course or treatment took place. In a few instances this was not possible, and the base-line and cortisone periods were not similar in all respects. Occasionally, three specimens could not be collected for each period, and base-line or cortisone figures are based on one or two determinations. With one exception, all patients were hospitalized during the study.

The 17-ketosteroid determinations were performed in triplicate by a modification of the method of Masuda and Thuline,[5] using the Zimmerman reaction. Determinations on all six urine specimens were usually performed on the same day, under identical conditions, by a technician who did not know the patient's diagnosis.

Studies have been completed on eighteen patients. Six of them had carcinoma of the prostate. Different stages of this disease were represented;

* Supported in part by an institutional research grant from the American Cancer Society.
Received for publication March 18, 1954.

Copyright 1954 by THE YALE JOURNAL OF BIOLOGY AND MEDICINE, INC.

for example, in one case the disease was sufficiently localized to warrant treatment by radical prostatectomy; two presented extensive bone metastases, pulmonary involvement, and anemia. Eight patients were "controls" without neoplastic disease. Three of these were essentially well individuals undergoing rehabilitation from paralytic poliomyelitis. The others were chronically ill patients. Four patients had inoperable malignant disease not known to be primary in the prostate. One of the "control" patients was a young woman; all other patients were men. Table 1 summarizes the major features of these eighteen case histories.

RESULTS

The 17-ketosteroid determinations on this group of eighteen patients are presented in Table 2. The base-line values fell within the usual range and varied as would be expected with sex and age, except that several of the patients with carcinoma of the prostate excreted somewhat larger amounts than usual for elderly men. In general, there was relatively little variation among the three base-line urine specimens from any one patient.

There was considerable variation in 17-ketosteroid excretion during the first day cortisone was administered. In some cases it was close to the base-line figure; in others it was in the same range as the excretion during the second and third day. Because of the variability, this figure was not used in making comparisons between different groups of patients.

During the second and third cortisone days the patients without malignant neoplasms excreted somewhat larger amounts of 17-ketosteroids than during the base-line period. The average increment over the base line for this group of eight patients was 4.3 mg. per 24 hours. Individual values ranged from a decrement of 2.3 mg. to an increment of 11.7 mg. In one patient the excretion on the third day exceeded that on the second day by 5 mg. or more; in another, the reverse was true. The averages for the second and third days were not significantly different.

Considerable increase in 17-ketosteroid excretion during the second and third days of cortisone administration was noted in all six patients with carcinoma of the prostate. The average increment over the base-line for this group was 21.8 mg. per 24 hours. This was five times the figure for the "control" group. Individual increments ranged from 9.1 mg. to 34.7 mg. The smallest average increment for any patient was 17.1 mg. In three patients the excretion on the third day exceeded that on the second by 10 mg. or more. In no patient did the second day's excretion significantly exceed the third. The average for the third day was appreciably higher than the second. These figures suggest that, in contrast to the patients with non-

TABLE 1. PATIENTS. DIAGNOSIS AND CLINICAL DATA

Case	*Sex*	*Age*	
1	F	22	Paralytic poliomyelitis two months before test. Undergoing physiotherapy and retraining. Urinary tract infection.
2	M	24	Degenerative cerebellar disease of long duration. Undergoing physiotherapy and retraining.
3	M	38	Paralytic poliomyelitis two years before test. Undergoing physiotherapy and retraining. Flail legs, arthritis of hips and spine, palpable liver and spleen, chronic sinusitis.
4	M	80	Benign overgrowth of prostate, symptomatic. Incomplete emptying of bladder, urinary tract infection, severe arteriosclerosis, senility.
5	M	61	Multiple cerebral thromboses. Semicomatose. Suprapubic cystostomy. Urinary tract infection, malnutrition, decubiti, fever. (On Danger List.)
6	M	73	Anemia, hypo-albuminemia, weight loss, joint pain. Benign overgrowth of prostate, bilateral hydronephrosis, degenerative arthritis of hip, slight fever. Inlying catheter.
7	M	29	Paralytic poliomyelitis one year before test. Undergoing physiotherapy and retraining.
8	M	43	Multiple sclerosis of long duration, with mental deterioration. Elevated spinal fluid protein.
9	M	72	Carcinoma of the prostate. Orchiectomized; on stilbestrol. Local extension of tumor.
10	M	66	Carcinoma of the prostate. Orchiectomized; on stilbestrol. Local extension of tumor, multiple osteoblastic bone metastases, pulmonary infiltration, anemia. Elevated acid and alkaline phosphatase.
11	M	74	Carcinoma of the prostate. Radical prostatectomy five days before test. Arthritis.
12	M	74	Carcinoma of the prostate. Orchiectomized; on stilbestrol. Multiple osteoblastic bone metastases, pulmonary infiltration, anemia. Normal phosphatases.
13	M	76	Carcinoma of the prostate. Orchiectomized; on stilbestrol. Multiple osteoblastic bone metastases.
14	M	59	Carcinoma of the prostate. Orchiectomized; on stilbestrol. Local extension of tumor, urinary symptoms, edema of one leg.
15	M	24	Hodgkin's disease. Fever, anemia, pleural effusions, bone lesions.
16	M	74	Metastatic carcinoma of bone, primary site unknown. Benign overgrowth of prostate. Elevated alkaline phosphatase.
17	M	53	Epidermoid carcinoma of bladder. Invasion of prostate, ureteral obstruction. Phosphatases normal.
18	M	68	Carcinoma of the head of the pancreas, lymph node metastases. Laparotomy and cholecystojejunostomy seven days before test. Obstructive jaundice, two months.

malignant disease, steroid excretion was still rising on the third cortisone day among the patients with prostatic cancer.

Three of the patients with other neoplasms reacted like the group with prostatic cancer. The fourth, Case 18, reacted like the controls. It may be significant that this patient had impairment of liver function.

DISCUSSION

The difference between the responses of patients without malignant disease and those with cancer of the prostate is quite striking. As is shown

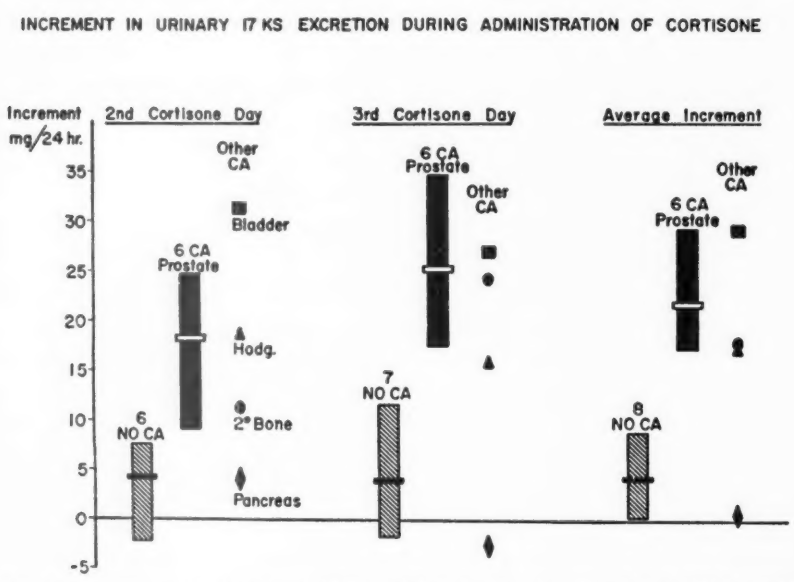


FIG. 1. Increments in 17-ketosteroid excretion during the second and third cortisone days. The columns represent the range of values for the respective groups of patients; each horizontal line across a column represents the mean value for that group.

in Figure 1, there is no overlap between the two groups on either the second or the third cortisone day, and the mean increment in 17-ketosteroid excretion in the latter group is more than five times that of the former. It is possible that the difference would have been even greater had the experimental period been longer. The data suggest that 17-ketosteroid excretion was still rising on the third cortisone day in some patients with prostatic carcinoma; this did not seem to be true of the non-malignant group.

There are relatively few data in the literature on 17-ketosteroid excretion by patients receiving doses of cortisone comparable to those used in this study. Burstein, Savard, and Dorfman[1] studied the urinary steroids ex-

TABLE 2. 17-KETOSTEROID DETERMINATIONS

Case	Base-line period				Cortisone period					
								Increment over average Base-line		
	1st day	2d day	3d day	Average	1st day	2d day	3d day	2d day	3d day	Average
	mg./24 hrs.				mg./24 hrs.					
				NON-MALIGNANT NEOPLASM						
1	5.4	6.4	5.4	5.7	9.9	13.3	15.9*	7.6	10.2*	8.9
2	8.7	7.3	8.1	8.0	10.3	15.4	16.9	7.4	8.9	8.2
3	21.4	13.5	22.9	19.3	12.9	25.3	19.6	6.0	0.3	3.2
4	6.8	5.9	...	6.4	...	7.6	...	1.2	...	1.2
5	3.3	3.2	3.9	3.5	2.9	8.5	9.1	5.0	5.6	5.3
6	7.2	7.1	6.3	6.9	10.3	9.4	5.2	2.5	—1.7	0.4
7	12.5	15.4	13.4	13.8	15.2	...	16.4	...	2.6	2.6
8	22.2	21.2	17.1	20.2	17.5	17.9	31.9	—2.3	11.7	4.7
							Mean	3.9	5.4	4.3
				CARCINOMA OF PROSTATE						
9	16.8	4.7	9.3	10.3	...	30.4	27.8	20.1	17.5	18.8
10	8.2	8.0	8.7	8.3	9.9	31.9	43.0	23.6	34.7	29.2
11	14.6	13.0	9.0	12.2	26.0	29.0	39.5	16.8	27.3	22.1
12	3.4	7.4	1.4	4.1	4.9	13.2	29.1	9.1	25.0	17.1
13	12.2	14.0	12.6	12.9	27.8	28.5	32.6	15.6	19.7	17.7
14	11.7	13.3	10.3	11.8	16.4	36.6	39.7	24.8	27.9	26.4
							Mean	18.3	25.3	21.8
				OTHER NEOPLASMS						
15	8.2	9.4	10.3	9.3	12.1	27.9	25.1	18.6	15.8	17.2
16	12.7	14.6	12.6	13.3	16.2	24.7	37.7	11.4	24.4	17.9
17	6.3	6.1	5.5	6.0	25.0	37.4	33.0	31.4	27.0	29.2
18	5.9			5.9	13.4	10.2	3.3	4.3	—2.6	0.9

* Third-day specimen lost. Fourth-day specimen used.

creted by an essentially healthy man who ingested 2,500 mg. of cortisone over a five-day period. Calculations from their data indicate that there was no rise in 17-ketosteroids during this period. Sandberg, *et al.*[6] noted a small increment in the 17-ketosteroid excretion of a 42-year-old man with rheumatoid arthritis during a course of therapy with 300 mg. of cortisone daily. Maddock, Chase, and Nelson[4] determined 17-ketosteroid excretion of four male patients with rheumatoid arthritis who were receiving 500-800 mg. of cortisone daily by intramuscular injection. They reported increases of 3 to 13 mg. per 24 hours over base-line excretion. The findings in these three studies are consistent with our "non-malignant" response.

Valk and Owens[7] and Harrison, Thorn, and Jenkins[3] indicated that administration of large doses of cortisone to patients with carcinoma of the prostate resulted in increased 17-ketosteroid excretion. Figures pertinent to our study are given for only one patient—Case 3 of the latter report. This patient's 17-ketosteroid excretion rose from 6 to 47 mg. per 24 hours after five days of administration of hydrocortisone at a dose of 300 mg. per day. This is consistent with our "positive" response.

Discussion of the mechanisms involved in the production of these differences in ketosteroid excretion must await the accumulation of additional data. Several conclusions may be drawn from this preliminary study. These differences do not reflect the over-all clinical status or nutritional balance of the patients. Two of the patients with prostatic carcinoma were in excellent condition except for their local disease. The sickest patient in this series, on the other hand, was Case 5, a "control." Non-malignant disease of the prostate did not produce a "positive" response. There was no correlation with acid phosphatase, alkaline phosphatase, or the presence or absence of demonstrable bone involvement.

The sensitivity of this response is not known. None of the patients in this series could be said to have "early" or "questionable" cancer, although two had disease of relatively limited extent. Of considerable interest is the question whether the phenomenon under study is especially related to prostatic malignancy, or whether it is common to many forms of cancer. Studies now under way should shed light on this and other questions, which largely determine the usefulness of a "cancer test."

The cortisone "test" was ordinarily performed only upon patients whose diagnoses were known. An interesting exception was Case 16. This 74-year-old man was admitted in a semicomatose state, with fever, stiff neck, and a type III pneumococcal bacteremia. He improved gradually on intensive antibiotic therapy. X-ray examinations revealed increased densities in the ribs, spine, pelvis, and femora. Clinical opinion was divided, with some

observers favoring the diagnosis of metastatic carcinoma of bone, and others, Paget's disease. Repeated careful examinations revealed no primary site of carcinoma. Two bone marrow aspirations and a needle biopsy of the prostate were negative. However, the 17-ketosteroid excretion rose sharply during cortisone administration. Largely on the strength of this finding, surgical biopsy of a rib was performed, and metastatic carcinoma was demonstrated. The primary site has not yet been discovered.

SUMMARY

The effect of large doses of cortisone on 17-ketosteroid excretion was studied in patients with and without malignant neoplastic disease. Eight patients without cancer showed little or no increase in urinary 17-ketosteroids while receiving 300 mg. of cortisone acetate per day. Six patients with carcinoma of the prostate showed a marked increase in 17-ketosteroid excretion under similar conditions of cortisone administration. There was no overlap between these two groups. Three of four patients with other malignancies showed a response similar to that of the patients with prostatic cancer. The possibility that this phenomenon may have some clinical usefulness as a "cancer test" is being investigated.

REFERENCES

1 Burstein, S., Savard, K., and Dorfman, R. I.: The in vivo metabolism of cortisone. Endocrinology, 1953, *52,* 448.

2 Deming, C. L., Harvard, B. M., and Sokal, J. E.: Experiences with the use of cortisone in the management of far advanced carcinoma of the prostate. (In preparation.)

3 Harrison, J. H., Thorn, G. W., and Jenkins, D.: Total adrenalectomy for reactivated carcinoma of the prostate. N. England J. M., 1953, *248,* 86.

4 Maddock, W. O., Chase, J. D., and Nelson, W. O.: The effects of large doses of cortisone on testicular morphology and urinary gonadotrophin, estrogen and 17-ketosteroid excretion. J. Laborat. Clin. M., 1953, *41,* 608.

5 Masuda, M. and Thuline, H. C.: An improved method for determination of urinary 17-ketosteroids. J. Clin. Endocr., 1953, *13,* 581.

6 Sandberg, A. A., Nelson, D. H., Glenn, E. M., Tyler, F. H., and Samuels, L. T.: 17-Hydroxycorticosteroids and 17-ketosteroids in urine of human subjects: Clinical application of a method employing β-glucuronidase hydrolysis. J. Clin. Endocr., 1953, *13,* 1445.

7 Valk, W. L. and Owens, R. H.: Effect of cortisone on patients with carcinoma of prostate. Tr. South Central Sec., Am. Urol. Ass., 1952, pp. 93-99.

ALFRED GOTTSCHALK

The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

THE INFLUENZA VIRUS ENZYME AND ITS MUCOPROTEIN SUBSTRATE*

With regard to size and enzymatic equipment viruses may be regarded as representing the lowest level of life. Their size ranges from about 20 to 400 mμ. Viruses do not grow on cell-free culture media; they multiply in living cells, and only those cells are host cells whose membranes have suitable adsorption areas for attachment of the virus particle and whose metabolic processes can be directed into such channels as to provide the material resources for the replication of virus genetic patterns. It is probably true to say that viruses have nothing like the elaborate enzymic equipment of even the smallest bacteria; however, the statement that viruses are devoid of enzymes is an oversimplification and certainly does not hold for the influenza virus. Most intracellular enzymes have been discovered in the past by allowing well-defined compounds, known to be metabolized by the living cell concerned, to be acted upon by an appropriate extract of the cell and then to investigate the chemical change induced, the variation of the reaction rate with temperature, pH, ionic environment, etc. An enzyme will remain undetected when no suggestion is available about the possible nature of its substrate, since no sensible plan can be devised to test for the unknown activity of an enzyme.

The first observation relevant to the existence of a viral enzyme was made by Hirst.[27] He found that influenza virus is adsorbed to the surface of erythrocytes and by forming bridges between the cells agglutinates them. The existence of large aggregates underlying the pattern formation in influenza virus haemagglutination implies that the virus particles and erythrocytes must be multivalent with regard to each other. Virus haemagglutination, being an adsorption phenomenon, proceeds readily at 0° C. If the red cells with the influenza virus particles attached to them are brought to 37° C., spontaneous elution of the virus into the medium takes place.[28] The virus present in the medium is functionally intact and available for further haemagglutination; the red cells, however, are irreversibly changed, having lost their ability to adsorb virus. Hirst[28] interpreted this phenomenon in terms of an enzyme-substrate interaction, the enzyme possessed by the virus acting upon a receptor substance present at the surface of the red cell, an

* This article is based on a paper given as Seminar in Microbiology at the Yale University School of Medicine, November 6, 1953.

Received for publication January 29, 1954.

interaction followed by separation of the enzyme from the reaction products. This interpretation was supported by Burnet's discovery[9, 10] of a soluble enzyme in cultures of vibrio cholerae. The vibrio enzyme was shown to imitate almost to the last detai lthe effect of the virus on the red cell receptors.[40] Other observations were reported, all of them difficult to understand on any other basis than on Hirst's concept of virus enzyme—cellular receptor interaction. Briody,[5] for instance, found that influenza B virus upon heating to 55° C. for 30 minutes is still adsorbed to and agglutinates red cells, but fails to elute spontaneously. Hanig[20] and Ada and Stone[2, 43] demonstrated a decrease in the electrophoretic mobility of human erythrocytes after treatment with influenza virus, different strains reducing the mobility value to different levels. The largest decrease in electrophoretic mobility was experienced when the red cells were treated with a purified preparation of the enzyme obtained from vibrio cholerae cultures referred to as receptor destroying enzyme (RDE). That both influenza virus and RDE are acting upon the same chemical groupings of the receptor substance embedded in the cell surface is indicated by the lack of any summation effect on the electrophoretic mobility when RDE treatment of the erythrocytes is preceded by treatment with influenza virus.[43]

The same sequence of events described for the system influenza virus and erythrocytes was observed when surviving host cells were substituted for erythrocytes. Thus influenza virus is adsorbed to and spontaneously elutes from the susceptible respiratory cells of the excised ferret[29] and mouse[18] lung. Pretreatment with RDE renders the respiratory surface of the excised mouse lung unable to adsorb influenza virus[18]; indeed, infection of the respiratory tract of the intact animal by inhaled influenza virus can be prevented when preceded by RDE treatment,[42] just as influenza virus infection of the chorio-allantoic membrane of the embryonated egg is hindered after the lining cells have been exposed to the action of RDE.[41] The similarity of the point of attack on cellular receptors of the soluble vibrio enzyme and the influenza virus particle could be demonstrated in many other ways; red cells, for instance, the receptors of which have been altered by one of the two agents fail to adsorb the other agent, and adsorption in the cold of one agent onto the intact erythrocytes blocks the adsorption of the other agent, as coating of the cells with one type of virus precludes the adsorption of a second type.[40] Furthermore, treatment with influenza virus as well as with the vibrio enzyme renders cells agglutinable by normal human sera to equivalent titres.[40]

Another test object for the nature of the agent present at the influenza virus surface and causing the loss of biological activity of certain cellular

receptors was provided by the observation of Francis[18] that heating influenza virus at 56° C. for 30 minutes allows normal serum to inhibit its haemagglutinin. Anderson,[3] in this Institute, discovered that the anti-haemagglutinin activity of normal serum is lost on treatment by living influenza virus or RDE. In addition to normal serum quite a number of secretions of human or animal origin (human meconium, ovarian cyst mucin, cervical mucus, respiratory mucus, sheep submaxillary glands mucin, hen egg-white) were found to contain a potent virus haemagglutinin inhibitor; again digestion with RDE or the infective influenza virus particle destroyed irreversibly the biological activity of the inhibitors.[8]

The above results clearly demonstrated that in all essentials the action of the influenza virus on red cell receptors, host cell receptors, and on haemagglutinin inhibitors is paralleled by an enzyme produced by cholera vibrio strains. The data confirmed in every respect Hirst's suggestion of the presence of an enzyme at the surface of the influenza virus particle. In accord with this concept were the further observations that the inactivation of purified ovarian cyst mucoid by influenza virus and by RDE, respectively, under given conditions approximates a first order reaction, the rate of reaction being proportional to the virus and RDE concentration respectively, and that both agents have a pH optimum of 5.7 to 7.0.[7] A further outcome of the experimental results was the conclusion that the soluble mucins inhibit haemagglutination by competing with the red cell receptors for the influenza virus particle, that the cellular receptors and mucins have some structural feature in common, and that it is this common grouping which serves the viral enzyme and RDE as substrate.[4]

Over the past six years this laboratory has made an effort to establish by chemical methods the activity of the viral enzyme, to elucidate the chemical grouping attacked by the enzyme, and to throw some light on the mode of the enzyme action. We restricted our work to the virus action on soluble mucoids inhibiting virus haemagglutination since the preparation of cellular receptors in quantity did not seem feasible, and in fact no electrophoretically homogeneous preparation of red cell receptors has been obtained so far in any laboratory. It was first shown that of the various components of hen egg-white only the ovomucin fraction is able to inhibit haemagglutination by heated influenza virus[22, 32] and that the inhibitory power of ovomucin is readily reduced both by living influenza virus and RDE.[22] From a reaction mixture of ovomucin and a small amount of highly purified influenza A virus, a dialysable compound was isolated consisting of carbohydrate and a nitrogenous substance.[23] This compound, referred to as split product, had quite unusual features. The carbohydrate was unstable even towards mild

acid treatment (1.0 N HCl, 100° C., 30 minutes) which caused formation of considerable amounts of soluble and insoluble humin substances. The nitrogenous component, after treatment with 0.1 N sodium carbonate for 12 minutes at 100° C., coupled with p-dimethylaminobenzaldehyde (p-dab) to give a purple colour identical with, but much more stable than, that produced by an authentic specimen of N-acetyl D-glucosamine; spectroscopically the respective colouring matters exhibited the same adsorption bands. However, in contrast to N-acetyl D-glucosamine the split product was stable to prolonged heating at 100° C. with 0.1 N sodium carbonate, yet unstable towards mild acid treatment. After heating for few hours with 1.0 N HCl the compound failed to give a colour with p-dab even after appropriate pretreatment with acetylacetone and alkali, whereas glucosamine arising from the acid hydrolysis of N-acetyl D-glucosamine readily gives the Ehrlich reaction after heating with acetylacetone in alkali. As in Hirst's experiments with red cells and virus, so was shown in the assay with ovomucin and virus that the virus, after reducing irreversibly the biological activity of the mucoprotein by more than 95%, was available for inactivation of another quantity of native ovomucin. A compound with the same properties was obtained when RDE instead of virus was taken as the catalytic agent.[23]

It was early recognised[15, 33] that ovomucin is a protein fraction consisting of the haemagglutinin inhibitory mucoprotein, one or more inert mucoproteins, and a small proportion of lysozyme. That the formation of the split product resulted from the interaction between living virus and the inhibitory mucoprotein was shown by the following controls, none of which yielded the dialyzable compound: (i) Heat-inactivated virus (55° C.; 30 minutes) and ovomucin; (ii) living virus and ovomucin, the inhibitory portion of which had been removed by selective adsorption.[23]

Though at this stage the chemical identification of the split product was unsatisfactory, its isolation did not leave any doubt as to the enzymic nature of the reaction investigated and as to the site of the enzyme. There is no other reasonable interpretation than that the enzyme is present at the surface of the virus particle; indeed, it seems to form part of this surface because it cannot be separated from the particle. That the enzyme is an integral part of the influenza virus was shown[24] by submitting the virus to three independent purification procedures; though by these methods up to 99 per cent of the nitrogen of the crude virus preparation was removed, the ratio haemagglutinin titre/enzymic activity remained unchanged. Moreover, the enzyme is not present in the normal host cell.[24]

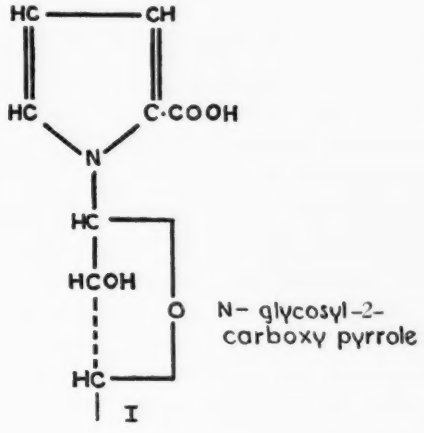
When an electrophoretically homogeneous urine mucoprotein inhibiting virus haemagglutination became available,[1, 37, 44] corresponding experiments with the pure material were carried out. It was gratifying to find[17] that coinciding with the loss of its biological activity upon influenza virus B action the pure urine mucoprotein released a dialyzable compound very similar in properties to that liberated from ovomucin. The yield was about 1 per cent of the mucoprotein used, and weight for weight the split product significantly exceeded in some assays the total virus used, the relevant figures in one experiment, for instance, being 8.5 and 5.0 mg. respectively. Examination of the split product revealed the presence of a single sugar and of a nitrogenous component, most probably an amino-acid. The split product, in its sensitivity towards mild acid treatment on heating, closely resembled amino-acid N-glucosides and their isomers (N-substituted isoglucosamines) which we had synthesized[18]; in these condensation products, e.g. between glucose and phenylalanine, the sugar moiety is readily transformed into 5-(hydroxymethyl)-2-furaldehyde by 2 N acetic acid (100° C., 2 hours) probably by virtue of the basicity of the NHR group attached to carbon atom one which favours the formation of the corresponding conjugate acid.

The purple colour that the split product, after alkali treatment, gave with p-dab remained unexplained since the properties described excluded N-acyl hexosamine as component and since the synthetic condensation products did not react with Ehrlich's reagent in the same manner. All observations pointed to the presence in the enzymatically released product of a hitherto unknown acid-labile and alkali-stable nitrogenous substance as being responsible for the Ehrlich reaction. This conclusion was strongly supported by the findings of Odin[36] that the split product reacted directly with p-dab, i.e., without any pretreatment, and that this direct Ehrlich reaction was also given by a number of influenza virus haemagglutinin inhibitory mucins. Odin's report prompted an investigation in our laboratory into the chemistry of the substance reacting directly with the p-dab reagent. We have recently isolated[20] this substance from an electrophoretically homogeneous inhibitory mucoprotein of bovine submaxillary glands and identified it as 2-carboxy-pyrrole on the following evidence: (i) Authentic 2-carboxy-pyrrole, prepared according to Oddo,[35] and the unknown component, obtained from the mucoprotein by mild alkali hydrolysis and extraction of the hydrolysate with ethanol, gave similar purple colours with p-dab instantly in the cold and for both the ninhydrin and Molisch reactions were negative. (ii) Paper partition chromatography, using phenol, butanol acetic acid, s-collidine, and butanol pyridine water, respectively, as

solvent and Ehrlich reagent as spray, revealed identical R_F values and colour shade for both substances. (iii) Reduction of the two substances with Adam's catalyst and hydrogen gas yielded a compound coinciding with DL-proline in R_F value and colour when run in the solvents mentioned and sprayed with isatin. (iv) Both substances were found to have sharp absorption peaks in the ultraviolet at 263.5 mμ. The pyrrole derivative prepared from the mucoprotein has yet to be crystalled.

It may be mentioned that neither tryptophan nor any other known protein component reacts with the Ehrlich reagent under the conditions applied to give a purple colour. It seems, therefore, justified to correlate the positive direct Ehrlich reaction, when carried out with mucoproteins or portions thereof in 1.0 N HCl, with the presence in them of 2-carboxy-pyrrole. On this assumption we found[20] the new imino-acid in the homogeneous inhibitory mucoproteins from human urine[1] and sputum,[24] from bovine and sheep submaxillary glands,[12] in the non-inhibitory pure mucoprotein from human plasma,[39, 45] in the non-inhibitory ovomucoid, in the ovomucin fraction, and in the glycoproteins casein and ox-serum albumin. The imino-acid is not present in carbohydrate-free proteins like insulin and globin (from haemoglobin), nor could it be traced in the mucopolysaccharides heparin and hyaluronic acid, both of which contain hexuronic acid.[21]

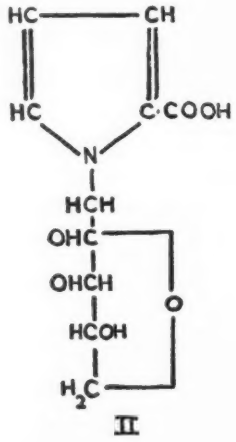
We could confirm Odin's observation that the split product gives the direct Ehrlich reaction. This quality taken together with the other properties of the split product,[17, 23] namely, the positive Molisch test, its reducing power in alkali medium, its nitrogen content, the instability towards mild acid treatment both of the sugar moiety and of the component coupling directly with p-dab, and the alkali stability of the latter is best explained by the structure I:



I

The alkali stability of pyrrole and the instability of pyrrole carboxylic acid towards acid are known, and the humin formation of the split product upon heating with 1.0 N HCl is closely imitated by a mixture of 2-carboxypyrrole and a reducing hexose or pentose when submitted to the same conditions.[21]

Recently Klenk and Lauenstein[31a] have prepared from bovine submaxillary glands and from human urine the crystalline methoxy derivative of "neuraminic acid"; the analytical data from the crystalline material are in close agreement with the formula $C_{11}H_{21}O_9N$; its structure is unknown. Neuraminic acid resembles the split product in its reducing power, its direct reaction with Ehrlich's reagent, and in its strong humin formation on heating with dilute mineral acid. If, as the authors assume, the two compounds are identical, diagram II may be suggested as the common structure:



II

Structure II is the Amadori rearranged isomer of structure I. The methoxy derivative of structure II would have the formula $C_{11}H_{21}O_9N$, when the compound crystallizes with three molecules of water.

Treatment of cellular receptors and inhibitory mucoproteins with periodic acid at low concentration modifies within a short time these substances in such a way as to make them no longer a suitable substrate for the viral enzyme.[6, 14, 20, 21] This modification was ascribed to the well-known oxidation of α-glycol groups in carbohydrates and interpreted to mean that the carbohydrate moiety of mucoproteins is the preferential site of attack by the viral enzyme. The presence in the split product of a carbohydrate strengthened this view. An analysis for carbohydrate components of the acid hydrolysate of the homogeneous urine mucoprotein was, therefore, carried out[19] with

the following result: 5.4 per cent galactose, 2.7 per cent mannose, 1.0 per cent fucose, and 7.6 per cent hexosamine; the hexosamine present was shown to be a mixture of glucosamine and galactosamine. Odin,[20] in an independent investigation on the same subject, reported the following figures: 8.1 per cent total hexose (galactose and mannose), 1.0 per cent fucose, and 7.6 per cent hexosamine. As mentioned above, the urine mucoprotein also contains 2-carboxy-pyrrole, the quantitative estimate amounting to about 4 per cent. By treatment of the inhibitor with 10 per cent barium hydroxide at 100° C. for several hours, the carbohydrate moiety may be separated from the bulk of the protein. On acid hydrolysis the polysaccharide yielded galactose, mannose, fucose, and hexosamine in proportions similar to those quoted above for the acid hydrolysate of the total mucoprotein.[19, 21] In addition to the sugars the polysaccharide contained the iminoacid.[20] In order to obtain some information about the size of the polysaccharide, the compound after separation from the protein by alkali treatment was submitted to dialysis through a cellophane membrane with lysozyme (molecular weight 16,000) as control. The polysaccharide dialysed slowly through a membrane of a pore size which allowed lysozyme to pass to a minute degree only.[19]

The composition of the polysaccharide, assuming that about 75% of the total 2-carboxy-pyrrole are incorporated in it, may be summarized as follows:

6 galactose residues 3 mannose residues 1 fucose residue 8 hexosamine residues 5 2-carboxy-pyrrole residues	molecular weight $(3488)_n$ where $n < 5$

The molecular weight of the polysaccharide, assuming that 3 hexosamine residues (see below) are N-acetylated, is about $(3500)_n$. The dialyzability through a cellophane membrane retaining lysozyme would suggest that n is not more than three or four. We regard[19] the mixed polysaccharide of a molecular weight of the order of 10,000 as an individual prosthetic group and visualize the mucoprotein as a conjugated protein containing as prosthetic group a relatively small polysaccharide; about 150 of such polysaccharides may be linked to each protein on the basis of a molecular weight[44] of 7×10^6 for the urine mucoprotein and of a carbohydrate content including 2-carboxy-pyrrole of about 20 per cent. The concept of multiple small-sized carbohydrate prosthetic groups accounts much better for the biological and biophysical data than the presence in the mucoprotein of a

giant polysaccharide, molecular weight 1.4 x 10^6, linked through a single bond with the protein moiety.

It seems of interest that the carbohydrate complex (prosthetic group) of the electrophoretically homogeneous inhibitory mucoprotein prepared from human sputum consists of galactose, fucose, hexosamine, and 2-carboxy-pyrrole.[21]

There is some indication that the polysaccharide in the inhibitory urine mucoprotein is of a branched structure. Assuming the presence in the mucoprotein (molecular weight 7 x 10^6) of 150 prosthetic groups in the form

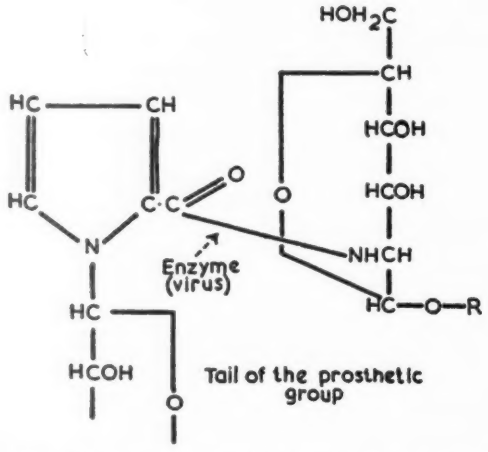


FIG. 1. Showing the viral enzyme susceptible bond between the carboxyl of pyrrole and the amino group of the D-galactosamine.

of linear polysaccharides and an average molecular weight of the split product (N-glycosyl-2-carboxy-pyrrole) of 250, the removal by viral enzyme action of even all the terminal N-glycosyl-2-carboxy-pyrrole groupings would account for a yield of not more than 0.5 per cent referred to dry matter of the substrate added. The experimental yield on split product, however, is about 1 per cent. It seems, therefore, more likely that the polysaccharide is of the branched type having perhaps three or four terminal N-glycosyl-2-carboxy-pyrrole groups. It may well be that the degree of branching is one of the factors determining the affinity between a given strain of influenza virus and the various inhibitory mucoproteins having polysaccharides of similar composition.

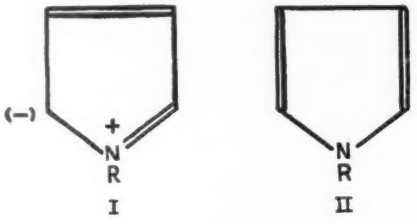
With regard to the linkage of 2-carboxy-pyrrole within the prosthetic group of the inhibitory urine mucoprotein it is suggested[20] that its carbonyl

carbon forms an amide bond with the nitrogen of an adjacent hexosamine, and that the pyrrole nitrogen engages in a glycosidic linkage with a sugar residue. The pyrrole nitrogen has no basic property; hence, if the viral enzyme be an amidase (see Fig. 1), breaking the susceptible carbon-nitrogen bond, its action will add a positive charge to the residual mucoprotein not counterbalanced by an equivalent loss in the split product. In fact, it has been shown by Horsfall[37] for the urine mucoprotein and by Pye[38] in this Institute for all inhibitory mucoproteins prepared so far in a homogeneous state that their net negative charge sharply decreases after treatment with influenza virus or with RDE, as it was previously found for the red cells.[2] This decrease in net negative charge could be due to either the loss of a negatively charged group or the unmasking of a positively charged one. Recent acid-base titrations showed that amino groups of pK value 10.8 to 11.2 are uncovered by the action of Lee influenza virus or RDE on urine mucoprotein.[11]

From the reported results it may be concluded that the influenza virus is equipped with an enzyme the substrate of which is the prosthetic group (polysaccharide) of a conjugated protein. There are two or more enzymatically active spots at the virus surface spaced in such a way as to fit the terminal groupings of a heterogeneous, probably branched, polysaccharide. The polysaccharide consists of hexoses, methylpentose, hexosamines, and 2-carboxyl-pyrrole. There is good evidence that the compound released by viral enzyme action from the prosthetic group of the inhibitory urine mucoprotein consists of 2-carboxy-pyrrole and a single sugar linked probably in an N-glycosidic fashion, and there is some indication that the susceptible bond is an amide link between the carbonyl group of the pyrrole derivative and the amino group of an adjacent hexosamine.

No definite statement can be made about the manner in which the polysaccharide is joined to the matrix of the protein. It is tempting to speculate that the imino-acid 2-carboxy-pyrrole is present not only at the free end of the prosthetic group but provides also the chemical link between the amino group of a polypeptide and the reducing end of the polysaccharide, its carboxyl group forming a peptide bond with the amino group of an amino-acid and its nitrogen engaging the reducing carbon of the polysaccharide in a glycosidic linkage. This suggestion would account for the presence of the imino-acid in neutral mucoproteins and its absence in carbohydrate-free mucoproteins and in mucopolysaccharides containing acidic sugar derivatives; moreover, it would bring the carbohydrate-protein link in line with the type of bond joining in nucleosides and co-enzymes the pentose or desoxypentose component with the respective purine, pyrimidine, and pyri-

dine derivative. In all these natural compounds the basic nitrogen of a heterocyclic ring engages the 5 carbon sugar in a N-glycosidic linkage; it may be of interest in this context that some pyrrole derivatives react according to a polar pyrrole structure I and the imine structure II[46]:



REFERENCES

1 Ada, G. L. and Gottschalk, A.: Preparation from urine of a pure substrate (mucoprotein) for the influenza virus enzyme. Austral. J. Sc., 1952, *14*, 160.

2 Ada, G. L. and Stone, J. D.: Electrophoretic studies of virus—red cell interaction: Mobility gradient of cells treated with viruses of the influenza group and the receptor-destroying enzyme of V. cholerae. Brit. J. exp. Path., 1950, *31*, 263.

3 Anderson, S. G.: Mucins and mucoids in relation to influenza virus action. I. Inactivation by RDE and by viruses of the influenza group of the serum inhibitor of haemagglutination. Austral. J. Exp. Biol., 1948, *26*, 347.

4 Anderson, S. G., Burnet, F. M., Fazekas de St. Groth, S., McCrea, J. F., and Stone, J. D.: Mucins and mucoids in relation to influenza virus action. VI. General discussion. Austral. J. Exp. Biol., 1948, *26*, 403.

5 Briody, B. A.: Characterization of the enzymic action of influenza viruses on human red cells. J. Immun., Balt., 1948, *59*, 115.

6 Burnet, F. M.: Mucins and mucoids in relation to influenza virus action. III. Inhibition of virus haemagglutination by glandular mucins. Austral. J. Exp. Biol., 1948, *26*, 371.

7 Burnet, F. M.: Mucins and mucoids in relation to influenza virus action. V. The destruction of "Francis inhibitor" activity in a purified mucoid by virus action. Austral. J. Exp. Biol., 1948, *26*, 389.

8 Burnet, F. M.: Haemagglutination in relation to host cell-virus interaction. Annual Rev. Microbiology, 1952, *6*, 229.

9 Burnet, F. M., McCrea, J. F., and Stone, J. D.: Modification of human red cells by virus action. I. The receptor gradient for virus action in human red cells. Brit. J. Exp. Path., 1946, *27*, 228.

10 Burnet, F. M. and Stone, J. D.: The receptor-destroying enzyme of V. cholerae Austral. J. Biol., 1947, *25*, 227.

11 Curtain, C. C.: The nature of the bond split by the influenza virus enzyme. Austral. J. Exp. Biol., 1953, *31*, 623.

12 Curtain, C. C.: The purification of an inhibitor of PR8 influenza virus from bovine submaxillary glands. (In preparation).

13 Fazekas de St. Groth, S.: Destruction of influenza virus receptors in the mouse lung by an enzyme from V. cholerae. Austral. J. Exp. Biol., 1948, *26*, 29.

14 Fazekas de St. Groth, S.: Modification of virus receptors by meta-periodate. I. The properties of IO_4-treated red cells. Austral. J. Exp. Biol., 1949, *27*, 65.

15 Fazekas de St. Groth, S. and Gottschalk, A.: Ovomucin: A substrate for the enzyme of influenza virus. II. Demonstration of its inhomogeneity. Brit. J. Exp. Path., 1951, *32*, 21.

16 Francis, T.: Dissociation of the haemagglutinating and antibody-measuring capacities of influenza virus. J. Exp. M., 1947, *85*, 1.

17 Gottschalk, A.: N-Substituted isoglucosamine released from mucoproteins by the influenza virus enzyme. Nature, Lond., 1951, *167*, 845.

18 Gottschalk, A.: Some biochemically relevant properties of N-substituted fructosamines derived from amino-acid N-glucosides and N-arylglucosylamines. Biochem. J., 1952, *52*, 455.

19 Gottschalk, A.: Carbohydrate residue of a urine mucoprotein inhibiting influenza virus haemagglutination. Nature, Lond., 1952, *170*, 662.

20 Gottschalk, A.: Presence of 2-carboxy-pyrrole in mucoproteins and its relation to the viral enzyme. Nature, Lond., 1953, *172*, 808.

21 Gottschalk, A.: In preparation.

22 Gottschalk, A. and Lind, P. E.: Ovomucin, a substrate for the enzyme of influenza virus. I. Ovomucin as an inhibitor of haemagglutination by heated Lee virus. Brit. J. Exp. Path., 1949, *30*, 85.

23 Gottschalk, A. and Lind, P. E.: Product of interaction between influenza virus enzyme and ovomucin. Nature, Lond., 1949, *164*, 232.

24 Gottschalk, A. and Perry, B. T.: Ovomucin: A substrate for the enzyme of the influenza virus. III. Enzymic activity as an integral function of the influenza virus particle. Brit. J. Exp. Path., 1951, *32*, 408.

26 Hanig, M.: Electrokinetic change in human erythrocytes during adsorption and elution of PR8 influenza virus. Proc. Soc. Exp. Biol., N. Y., 1948, *68*, 385.

27 Hirst, G. K.: The quantitative determination of influenza virus and antibodies by means of red cell agglutination. J. Exp. M., 1942, *75*, 49.

28 Hirst, G. K.: Adsorption of influenza haemagglutinins and virus by red blood cells. J. Exp. M., 1942, *76*, 195.

29 Hirst, G. K.: Adsorption of influenza virus on cells of the respiratory tract. J. Exp. M., 1943, *78*, 99.

30 Hirst, G. K.: *Annual Report, The Rockefeller Foundation*, New York, International Health Division, 1945, p. 50.

31 Hirst, G. K.: The nature of the virus receptors of red cells. I. Evidence on the chemical nature of the virus receptors of red cells and of the existence of a closely analogous substance in normal serum. J. Exp. M., 1948, *87*, 301.

31a Klenk, E. and Lauenstein, K.: Zur Kenntuis der Kohlenhydratgruppen des Submaxillarismucins und Harnmucoproteids. Die Isolierung von Neuraminsäure als Splatprodukt. Zschr. phys. Chem., 1952, *291*, 147.

32 Lanni, F., Sharp, D. G., Eckart, E. A., Dillon, E. S., Beard, D., and Beard, J. W.: The egg white inhibitor of influenza virus haemagglutination. I. Preparation and properties of semi-purified inhibitor. J. Biol. Chem., 1949, *179*, 1275.

33 Lanni, F., Sharp, D. G., Ssáky, T. Z., and Beard, J. W.: The electrophoretic and biological complexity of ovomucin. Arch. Biochem., N. Y., 1950, *28*, 313.

34 Marmion, B. P., Curtain, C. C., and Pye, J.: The effect of human bronchial secretions (sputum) on the haemagglutinin and infectivity of influenza virus. Austral. J. Exp. Biol., 1953, *31*, 505.

35 Oddo, B.: Sull' iodiomagnesiopirrolo ed il suo impiego per la sintesi di composti pirrolici. Gazz. Chim. ital., 1909, *39*, 649.

36 Odin, L.: Carbohydrate residue of a urine mucoprotein inhibiting influenza virus haemagglutination. Nature, Lond., 1952, *170*, 663.

37 Perlmann, G. E., Tamm, I., and Horsfall, F. L.: An electrophoretic examination of a urinary mucoprotein which reacts with various viruses. J. Exp. M., 1952, *95*, 99.

38 Pye, J.: Assay of inhibitors of influenza virus haemagglutination by electrophoresis. (In preparation).

39 Smith, E. L., Brown, D. M., Weimer, H. E., and Winzler, R. J.: Sedimentation, diffusion and molecular weight of a mucoprotein from human plasma. J. Biol. Chem., 1950, *185,* 569.

40 Stone, J.D.: Comparison of the action of V. cholerae enzyme and of viruses on the red cell surface. Austral. J. Exp. Biol., 1947, *25,* 137.

41 Stone, J. D.: Prevention of virus infection with enzyme of V. cholerae. I. Studies with viruses of mumps-influenza group in chick embryos. Austral. J. Exp. Biol., 1948, *26,* 49.

42 Stone, J. D.: Prevention of virus infection with enzyme of V. cholerae. II. Studies with influenza virus in mice. Austral. J. Exp. Biol., 1948, *26,* 287.

43 Stone, J. D. and Ada, G. L.: Electrophoretic studies of virus-red cell interaction: Additive effect of viruses of the influenza group and the receptor-destroying enzyme of V. cholerae. Brit. J. Exp. Path., 1950, *31,* 275.

44 Tamm, I. and Horsfall, F. L.: A mucoprotein derived from human urine which reacts with influenza, mumps, and Newcastle disease viruses. J. Exp. M., 1952, *95,* 71.

45 Weimer, H. E., Mehl, J. W., and Winzler, R. J.: Studies on the mucoproteins of human plasma. V. Isolation and characterization of a homogeneous mucoprotein. J. Biol. Chem., 1950, *185,* 561.

46 Wibaut, J. P. and Guljé, A. R.: Ozonolysis of pyrrole and some of its homologs in connection with the structure of the ring system. Proc. Koninkl. Nederland. Acad. Wetenschap., 1951, *54*B, *330.* Chem. Abstr. 1953, *47,* 6934h.

DANIEL L. KLINE

Department of Physiology, Yale University School of Medicine

STUDIES ON THE PURIFICATION AND ACTIVATION OF PLASMINOGEN (PROFIBRINOLYSIN)*

With the availability of a method of purifying plasminogen from human Fraction III,[5] the therapeutic use of this enzyme in thrombotic conditions became a distinct possibility. Before attempting the clinical use of purified plasminogen, however, it appeared advisable to investigate the report[3] that the enzyme responsible for the fibrinolytic activity can be separated from the agent producing proteolysis since this would make available a more desirable preparation for animal and clinical use.

In addition, an investigation of the activation of plasminogen by streptokinase was considered desirable in view of the observation of Christensen[2] that when plasminogen is activated by streptokinase, the solution obtained contains only a small percentage of the lytic activity which appears if the activation is carried out in the presence of substrate. The convincing demonstration by Cliffton, *et al.*[4,5,6] that purified plasminogen, after activation by streptokinase in the absence of protective substrate, will dissolve clots in animals would make even more impressive the potent fibrinolytic power of this enzyme if Christensen's report is correct.

MATERIALS AND METHODS

Plasminogen (profibrinolysin) was prepared from plasma Fraction III kindly released for our use by the American National Red Cross and supplied through the generosity of Dr. Tillman Gerlough of E. R. Squibb and Sons. Purified profibrinolysin is stable in refrigerated acid solution (pH 2-4), as an air-dried powder, or it may be dried with alcohol and ether as suggested by Cliffton and Cannamela.[6]

Streptokinase (Lederle) was kindly supplied by Dr. Frank B. Ablondi of Lederle Laboratories. The preparation used contained 3,400 streptokinase units per mg. of powder.

Commercial soluble casein was reprecipitated once, washed with water, alcohol, and ether and stored *in vacuo*. For use, it was dissolved in phosphate-saline buffer and standardized to 5 per cent by Kjeldahl analysis.

---

* This study was supported in part by Institutional Grant 47D of the American Cancer Society.

Received for publication March 22, 1954.

Phosphate-saline buffer. 0.1 M sodium phosphate, pH 7.4, in 0.9 per cent sodium chloride. One ml. of 1:1000 merthiolate was added to each liter of solution.

Proteolytic activity was determined under the conditions described by Remmert and Cohen,[9] and their definition of a unit is used, e.g. that amount of enzyme producing an increase of 450γ of trichloroacetic acid (TCA) soluble tyrosine in a medium of 4 per cent casein between the 2d and 62d minutes. Tyrosine-like substances were estimated by determining the absorption of the TCA filtrates at 280 mμ. Remmert and Cohen have shown that the increase in this chromogenic material parallels the increase in TCA soluble nitrogen in this system.

Quantitative fibrinolytic assays were performed essentially according to the method of Christensen.[1]

TABLE 1. COMPARISON OF METHODS OF PREPARATION OF PLASMINOGEN (PROFIBRINOLYSIN) FROM 10 GMS. OF FRACTION III

Method	*Total N mg.*	*Proteolytic activity units*	*Specific activity P.U./mg. N*	*Fibrinolytic activity units*
A. Original[8]	15.0	553	37	0.8×10^6
B. Modified[8]	0.15	...	...	1.6×10^4
C. Direct addition of phosphate, pH 6.0	6.5	390	60	.....
D. Direct addition of phosphate, pH 8.5	6.0	385	64.2	.....

RESULTS

COMPARISON OF METHODS OF PREPARATION

A solution from 40 gms. of Fraction III was prepared as described in the original method up to the point of precipitation in the presence of phosphate. The solution was then divided into four equal portions which were treated as follows:

A—Dialyzed against 0.25 M phosphate, pH 6.0 for 10 minutes and transferred to the refrigerator. The precipitated enzyme was collected by centrifugation the following day and dissolved in 25 ml. of distilled water plus 2 drops of N HCl. This is the originally described procedure.[8]

B—Dialyzed against 0.15 M phosphate, pH 6.0 for 10 minutes and transferred to the refrigerator. The precipitated enzyme was collected by centrifugation the following day, carefully dispersed in 25 ml. of distilled water, and filtered through medium-fast filter paper. The use of 0.15 M

phosphate and the avoidance of the use of HCl to dissolve the final product are the modifications described by Cliffton and Cannamela.[3]

C—When analyses of the solutions obtained from the dialysis bags showed the phosphate to be .0002 in both A and B, 1 ml. of 0.02 M phosphate, pH 6.0, per 100 ml. of solution, was added directly to the third portion. The final pH was about 7.5. The precipitate obtained as in A and B was dissolved in 25 ml. of water plus 2 drops of N HCl.

D—The same as C except that the pH of the phosphate added was 8.5

The results obtained are shown in Table 1. It will be noted that only 1-2 per cent of the nitrogen and of the fibrinolytic activity was obtained

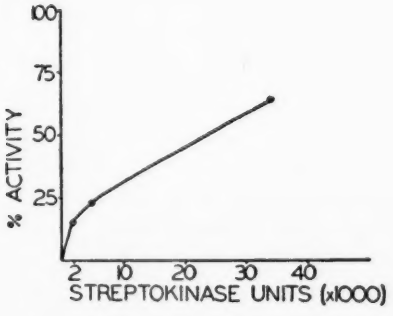


FIG. 1. The proteolytic activity of plasminogen after activation by varying amounts of streptokinase. The activity produced by 850 streptokinase units per mg. of enzyme in the presence of casein is designated as 100 per cent.

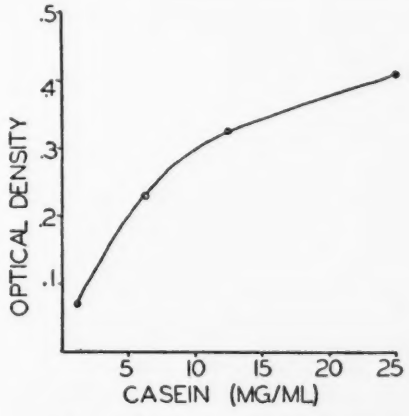


FIG. 2. The proteolytic activity of plasminogen after activation by 850 units of streptokinase per mg. of enzyme in the presence of varying concentrations of casein.

in experiment B (the modified method). The proteolytic activity of this weak solution was not measurable. When, instead of filtering the final precipitate prepared by the modified technique, it was dried with alcohol and ether, no difference in fibrinolytic or proteolytic activity was found as compared to material prepared by the original method. Upon dilution to the concentration of enzyme used by Cliffton and Cannamela (.0056 mg. N/ml.), proteolytic activity was not measurable, irrespective of the method of purification.

It will also be observed (Table 1, C and D) that the direct addition of phosphate resulted in a smaller yield of more highly purified material. At present, the pH of the final solution from which precipitation of the enzyme takes place is varied from 6.0 to 8.5 depending on whether maximum yield

or purity is desired. Above pH 8.0, the enzyme adheres tenaciously to glass surfaces.

THE ACTIVATION OF PLASMINOGEN BY STREPTOKINASE

When purified plasminogen was incubated at pH 7.4 with streptokinase, the activity obtained was far below that which appeared if casein were present during the activation (Fig. 1). Even with enormous amounts of streptokinase, full activity was not obtained. From Figure 2, it may be seen that the activity which appeared was directly related to the concentration of casein present during the activation. It may be noted in passing that the assay method of Remmert and Cohen calls for the presence of a casein concentration of 11.5 per cent during the activation. This concentration is not optimal and may explain the fact that when the enzyme is diluted, the proteolytic activity does not decrease proportionately.

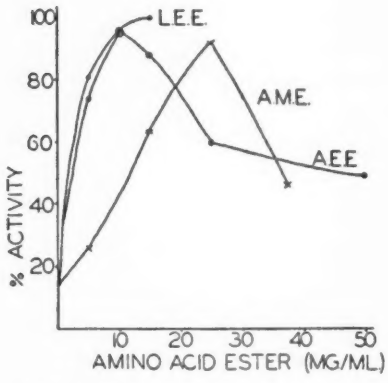


FIG. 3. The protective action of arginine methyl ester (x), arginine ethyl ester (o), and lysine ethyl ester (•) hydrochlorides. The activity produced by 850 units of streptokinase per mg. of enzyme is designated as 100 per cent.

Since the presence of casein renders the preparation unsuitable for repeated intravenous administration, other substrates of plasmin were tested for protective action. Troll, Sherry, and Wachman[10] have reported that certain esterified basic amino acids are split by plasmin. Therefore, arginine methyl ester (AME),* arginine ethyl ester (AEE), and lysine ethyl ester (LEE) were investigated. In carrying out the tests, the hydrochlorides of the ester compounds were dissolved in buffer, adjusted to pH 7-8 and brought to final volume before the addition of streptokinase powder. The plasminogen was dissolved at pH 3.5, and the solution was carefully adjusted to clarity at about pH 8.6. Distilled water was then added to bring the solution to the final concentration desired, usually 4 mg. per ml. Equal volumes of the two solutions were then mixed and incubated at 37.5° C. for 10 minutes. Aliquots, usually 0.4 ml. of the activated mixture, were added to 4 ml. of 5 per cent casein solution and sufficient buffer to make a final

* I am indebted to Dr. Joseph S. Fruton for a supply of AME; the other esters were synthesized by Mr. Jack Graff, Department of Biochemistry, Yale Medical School.

volume of 5 ml. Two ml. aliquots were withdrawn at 2 and 62 minutes and added to 2 ml. of 10 per cent TCA solution. The TCA precipitate was incubated at 37.5° C. for 10 minutes, 5 ml. of water were added, and the suspension was filtered through Whatman No. 3 filter paper.

The data shown in Figure 3, in which the activity obtained in the presence of casein is set at 100 per cent, demonstrate the protective action of these substances. The decline in protective action at high concentrations of the esters probably represents the effect of competition for the substrate. An additional experiment was performed to demonstrate the protective action of LEE in fibrinolytic tests. Two portions of a plasminogen solution were activated with streptokinase, one with and the other without LEE. The unprotected solution yielded 28.5 per cent of the proteolytic and 26.2 per cent of the fibrinolytic activity as compared to the protected solution.

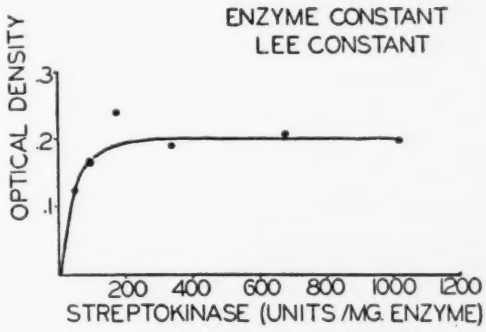


FIG. 4. The proteolytic activity of plasminogen after activation by varying amounts of streptokinase per mg. of plasminogen in the presence of 15 mg./ml. of lysine ethyl ester hydrochloride (.072M).

The arginine esters formed precipitates during the activation, and efforts to elute the enzyme from the precipitates proved fruitless. However, the precipitate formed with LEE was smaller in amount and could be removed with no loss of enzyme activity by centrifugation at pH 8.5. The final enzyme solution was water clear. The plasmin obtained was filterable through a Berkefeld filter but there was some loss in the process.

Using the optimal concentration of LEE hydrochloride, .072 M (15 mg./ml.), it was found, as is shown in Figure 4, that full activation of plasminogen in proteolytic tests was obtained with 2-300 units of streptokinase per mg. of enzyme (0.13 mg. of enzyme N). This amount of streptokinase produces little activated material in the absence of the protective compound (Fig. 1).

STABILITY OF PLASMIN

Plasmin solutions in the presence of LEE were stable for 24 hours at 4° C. and lost activity slowly thereafter until about 20 per cent remained after one week. Frozen solutions showed no loss of activity in two weeks.

TOXICITY STUDIES

Eleven mice were injected intravenously with 150 mg. of neutralized LEE hydrochloride per kg. of body weight.* This amount was calculated to be five times the quantity which will be used. No evidence of toxicity was obtained during a two-week observation period. At autopsy, all the organs appeared to be normal.

When dogs were injected intravenously with plasmin activated in the presence of LEE, the electrocardiogram was normal and blood pressure was unaffected.† When these studies are considered in conjunction with those of Cliffton, *et al.*,[4,5,6] it would appear that plasmin activated with streptokinase in the presence of LEE can safely be administered by the intravenous route. The observation of Cliffton, *et al.*[5] that small quantities of plasmin produce a temporary shortening of the clotting time, was confirmed.

DISCUSSION

We have been unable to confirm the claim that slight modifications of the original method of preparation of plasminogen produce a separation of a proteolytic from the fibrinolytic enzyme.[3] In addition to the evidence obtained earlier by Remmert and Cohen[9] and by Christensen and MacLeod[2] that the pH and temperature optimum for proteolytic and fibrinolytic activities and stability are identical, and that during purification the two activities increase proportionately, we have found that the degree of protection afforded by LEE is the same and that the lysis time is increased when a competing substrate, casein, is added to the enzyme system. On the basis of our studies, the failure of Cliffton and Cannamela to detect proteolytic activity in their preparations appears to have been the result of their using solutions which were too dilute to produce measurable activity in this type of assay. In adequate concentration, plasmin prepared by the modified method possessed the same specific proteolytic activity as the material obtained by the original method.

The observation that plasmin is rapidly destroyed in the absence of substrate has been described by Christensen.[1] The fact that the addition of substrate after the activation does not produce additional activity provides evidence that this is not a phenomenon involving the activation of plasminogen but involves protection of the active enzyme. Furthermore, when plasmin in the presence of substrate is precipitated and redissolved in water, there is an immediate destruction of the active enzyme.

---

* I should like to thank Dr. D. D. Bonnycastle for his assistance in the mice studies.
† Part of a study being conducted in conjunction with Dr. L. Nahum.

The demonstration of the dependence of protective action on the concentration of substrate may help to eliminate one of the variables which has made the assays of streptokinase and fibrinolysin somewhat erratic and has given rise to controversy concerning the kinetics of the activation process. In addition, it has been possible to show that the quantity of streptokinase needed for activation is considerably less than has hitherto been used. Since streptokinase is a somewhat toxic material,[2] this fact is of importance in the preparation of plasmin for biological use.

SUMMARY

1. It has been found that casein and basic amino acid esters will protect plasmin from destruction.

2. A method is described for the preparation of a plasmin solution which is stable and clear at a physiological pH. Toxicity studies with this preparation have been carried out. No harmful effects were obtained.

3. Evidence is presented which supports the conclusion that the proteolytic and fibrinolytic activities are manifestations of the actions of the same enzyme.

REFERENCES

1 Christensen, L. R.: Methods for measuring the activity of components of the streptococcal fibrinolytic system and streptococcal desoxyribonuclease. J. Clin. Invest., 1949, *28*, 163.

2 Christensen, L. R. and MacLeod, C. M.: A proteolytic enzyme of serum: Characterization, activation, and reaction with inhibitors. J. Gen. Physiol., 1945, *28*, 559.

3 Cliffton, E. E. and Cannamela, D. A.: Fibrinolytic and proteolytic activity of a human plasminogen prepared from Fraction III of human plasma. J. Appl. Physiol., 1953, *6*, 42.

4 Cliffton, E. E., Cannamela, D. A., and Grossi, C. E.: In vivo studies of human plasmin-intravenous injection in dogs and rabbits. J. Appl. Physiol., 1953, *6*, 143.

5 Cliffton, E. E., Grossi, C. E., and Cannamela, D. A.: Lysis of thrombi produced by sodium morrhuate in the femoral vein of dogs by human plasmin (fibrinolysin). Ann. Surg., 1954, *139*, 52.

6 Grossi, C. E., Cliffton, E. E., and Cannamela, D. A.: The lysis of intravascular thrombi in rabbits with human plasmin. Blood (in press).

7 Johnson, A. J. and Tillet, W. E.: The lysis in rabbits of intravascular blood clots by the streptococcal fibrinolytic system (streptokinase). J. Exp. M., 1952, *95*, 449.

8 Kline, D. L.: The purification and crystallization of plasminogen (profibrinolysin). J. Biol. Chem., 1953, *204*, 949.

9 Remmert, L. F. and Cohen, P. P.: Partial purification and properties of a proteolytic enzyme of human serum. J. Biol. Chem., 1949, *181*, 431.

10 Troll, W., Sherry, S., and Wachman, J.: Characterization of streptokinase activated plasminogen (plasmin) by synthetic substrates. Fed. Proc., Balt., 1952, *12*, 281.

WILLIAM HARRISON
AVERILL A. LIEBOW

Department of Pathology, Yale University School of Medicine

THE EFFECTS OF MASSIVE INTRAVENOUS INFUSIONS WITH SPECIAL REFERENCE TO PULMONARY CONGESTION AND EDEMA*

Some of the factors governing the development of edema in the lungs are understood in broad terms. These factors are considered to be no different from those operative in the systemic vessels—increased hydrostatic pressure, decreased oncotic pressure, excessive permeability of the vessels, and decreased lymphatic drainage. Little specific quantitative information is available relative to the pulmonary vasculature. Thus, although drainage from the lung by way of the lymphatics has been shown to increase during the development of pulmonary congestion and edema,[4] a study of the rôle of the lymphatics in the pathogenesis of edema has not been adequately approached. Anoxia is said to increase the permeability of the capillaries in the periphery, but its effect on those of the lungs is not known. It must also be remembered that other than capillary vessels participate in the exudative process; the rôle of the venules has recently been emphasized.[11] In the lung the protein osmotic pressure normally exceeds capillary hydrostatic pressure by a considerable margin, the opposite of the condition in the arterial ends of systemic capillaries. Thus it is possible to introduce large quantities of water via the trachea with very rapid absorption. Still adumbrated as are these various factors, it has become plain that they are interrelated and that pulmonary edema in no instance depends upon a single pathogenetic mechanism. For example, the very formation of the edema fluid involves some leakage of protein into the extravascular compartment. Also, as the alveoli become filled with exudate, an increasing barrier is interposed between the walls of the vessels and the oxygen of the atmosphere.

The complexities of these interrelationships became apparent in a study of the effects of increased intracranial pressure on the lesser circulation.[9] During these experiments, considerable quantities of saline fluid were introduced into the blood stream over a period of several hours, directly or via the subdural or subarachnoid space. The present observations are thus partly in the nature of a control study.

Previous studies of the effects of infusions of fluid have been variously interpreted. In part, contradictory statements have resulted from lack of

* Supported by a grant from the Office of Naval Research as a part of Project N6ori-44, Task Order XI.
Received for publication March 12, 1954.

uniformity in method, especially in regard to quantitative factors. The interpretations also vary depending upon the criteria employed for pulmonary edema; especially lamentable is the use of gross inspection as the sole criterion by some observers. Those reports where there is no knowledge of intravascular pressures, especially in the pulmonary capillaries, can be considered incomplete. Early work has been well reviewed by Warthen.[17]

Wiggers[18] has stated that he has introduced as much as 15 liters of saline into a medium-sized dog during the course of three hours without signs of the development of pulmonary edema; "if, however, pulmonary pressures are increased still farther by means of epinephrine, edema readily supervenes."

Warthen's[17] study was concerned with effects on electrolytes and erythrocyte fragility, but he did report, on the basis of gross inspection alone, the development of pulmonary edema in a dog receiving 3,700 cc. of dextrose and Ringer's solution within a 35-minute period. This represented an inflow rate of 672 cc./kg./hour. At post-mortem examination, thin fluid was found in the peritoneal cavity, but there was no free fluid within the pleura or pericardial sac.

Yeomans, Porter, and Swank[19] infused dogs under alpha-chloralose anesthesia at a rate of 150 to 300 cc./kg./hour over short intervals of time. The pulmonary venous pressure measured in one experiment rose to 440 mm. of water: this rise was more rapid and to a higher level than that in the peripheral veins. Rales were not heard in the lungs even at this high level. Under the same circumstances portal venous pressure also exceeded systemic venous pressure. The heart size was increased during the infusion. Cardiac output and oxygen consumption also were elevated. The serum proteins were reduced almost to half at rates of infusion ranging between 55 and 70 cc./minute.

Opdyke[18] and his co-workers, in recording simultaneously the pressures in the right and left atria, found that not only was the pressure usually higher normally on the left side in the open-chested dog, but they were also able to confirm Yeomans, Porter, and Swank's isolated observation that there was a relatively greater rise in the pressure within the left atrium when saline was introduced into the femoral vein. The fluid was introduced at a rate of 50 to 75 cc. a minute, and in a total volume varying between 200 and 500 cc.

Jenkins and his co-workers,[10] following the usage of Fegler and Banister[5] employed the term congestive atelectasis to describe a condition of dilatation of the capillaries with encroachment upon, or obliteration of the alveolar spaces. This they observed at certain high rates of infusion. In a preliminary

statement, they remarked that congestion and "stiffness" of the lung could develop while the left atrial pressure was falling. In some of their recorded experiments, the left atrial pressure was, however, found to rise more steeply than that in the right atrium.

An increased mortality over controls was demonstrated by Gibbon and Gibbon[6] when large infusions of plasma were given to cats after surgical removal of between two-thirds and three-fourths of the pulmonary substance.

In man, Gilligan, Altschule, and Volk,[7] and Altschule and Gilligan[1] found an appreciable increase in blood volume, but very little increase in venous pressure when the rate of infusion of saline was less than 20 cc./minute and the total volume was between 500 and 1,500 cc. When the rate of infusion was larger than the stated figure, venous pressure was significantly increased. Venous pressure returned to control levels within 10 to 25 minutes after interrupting the infusion.

Doyle and his co-workers[8] injected saline solutions into human subjects at rates of from 77 to 146 cc./minute, and in amounts of 950 to 1,000 cc. In each of 12 persons, the pulmonary arterial pressure increased continuously during the injection, and there was a rise in the "pulmonary capillary pressure," measured by the method of Dexter (considered as perhaps the same as the left atrial pressure), proportional to that observed in the pulmonary artery. The hematocrit fell on an average of 13% and there was an increase in the total blood volume of 14%. Cardiac output increased in half of the subjects, but not in the others. With the infusion of one liter of saline solution, there was no evidence of a disproportionately large rise in the pulmonary vascular pressures. It was suggested "that the changes introduced by rapid infusion can be explained most adequately on the basis of an abrupt, but not disproportionate increase in the amount of blood contained in the relatively indistensible pulmonary circuit."

In the present studies, saline solutions were injected into dogs at controlled rates over periods approximating five hours. Infusion rates were slower than in most previous experiments with canine subjects, but they were continued for longer periods of time. Thus they were somewhat closer to the conditions, albeit extreme, that might be expected in medical practice. Moreover, the thorax was intact, and the animals were not in an immediately postoperative state. Observations were made in several parameters—the pressure in the pulmonary capillaries, the total fluid retained in the intravascular compartment and elsewhere, and the urinary output. Furthermore, an attempt was made to employ objective criteria of pulmonary congestion and edema.

METHODS

Mongrel dogs between 7.7 and 21.3 kg. in weight were prepared with plastic needle guides[8] affixed surgically to the pulmonary artery and left atrium at least one week prior to the experiment. On the day of the experiment alpha-chloralose was administered intravenously, 25 mg./kg. body weight, supplemented with occasional small doses of sodium pentobarbitol when needed. Polyethylene catheters were introduced into the femoral vein, and through the femoral artery into the thoracic aorta. Their patency was maintained by filling them with heparin solution. Needles were inserted through the guides into the pulmonary artery and left atrium, and 0.9% saline at pH 7.0 and Tyrode's solution at pH 7.5 were introduced by gravity in equal volume at a rate regulated to give the calculated dose of fluid for any particular dog over a period approximating five hours. Through these taps, vascular pressures in the aorta (Ap), femoral vein (FVp), pulmonary artery (PAp), and left atrium (LAp) were recorded photokymographically every half hour during the course of the experiment with a bank of Hamilton manometers. This mixture of saline solutions was employed since it corresponded approximately to the fluids used in the cerebral compression experiments previously reported.

The procedure of introducing fluid at an equal rate into the pulmonary artery and left atrium was also as in this earlier work. Although the pressure recordings were made simply by switching the needle guides from the infusion jars to the manometers, there was no sag in the pressures during the 30 or more seconds of recording to indicate any striking displacement from equilibrium consequent to this technique. The pressures actually did not deviate strikingly from pre-infusion levels in Dog 3, an animal receiving the maximum quantity 23.1 cc. per minute, into each of the two ports. The bladder was catheterized so that the output of urine could be measured each half hour and its specific gravity noted. The hematocrit was followed, and in most instances this was supplemented at intervals by the determination of the carbon monoxide capacity of the blood by the method of Van Slyke and Hiller. In some dogs the oxygen content of the femoral arterial blood was measured early and late in the experiment. The scheme of grading the presence and degree of "pulmonary edema" was, according to the ratio of lung weight to body weight, as in our previous report,[9] where the deficiencies and advantages of this and other methods have been discussed:

1.20%: no pulmonary edema
1.21-1.40%: questionable pulmonary edema
1.41-1.60%: slight pulmonary edema
1.61-2.00%: moderate pulmonary edema
Over 2.00%: severe pulmonary edema.

In our experience as well as in that of Luisada and Sarnoff[12] the lung weight did not exceed 1.20% of the body weight in any normal animal.

OBSERVATIONS

In general, retention of fluid was proportional to fluid administered but this relationship was by no means constant, as indicated in Table 1. With infusion rates varying in different animals between 40 and 125 cc./kg./hour and total volumes of infusate ranging from 1,900 to 7,600 cc. over a period

of approximately five hours, the total urinary output ranged from as little as 163 cc. to as much as 4,428 cc. Expressed in terms of body weight and time, retention of fluid varied from 6 to 89 cc./kg./hour.

It is necessary to attempt a correlation of these observations, as well as of the degree of hemodilution, with the lung weight-body weight ratios

TABLE 1. LUNG WEIGHT-BODY WEIGHT RATIOS IN DOGS RECEIVING INFUSIONS OF SALINE SOLUTIONS (IN ORDER OF DECREASING AMOUNT OF FLUID RETAINED)

Dog no.	*B.W. kg.*	*Length cm.*	*Total fluid administered*	*Length of exp. (hr.)*	*Fluid infused cc./kg./hr.*	*Urine output cc.*	*Total fluid retained cc. kg./hr.*	*L.W./B.W. %*
1	11.3	89	7,035	5	125	2,000	89	2.02
2	13.2	92	7,600	5	115	2,417	78	1.21
3	21.3	98	6,800	3	103	1,940	76	1.32
4	13.2	88	5,400	5	82	765	70	1.97
5	11.3	88	5,250	5	93	1,475	67	1.96
6	12.7	94	6,275	5	98	2,097	66	1.15
7	7.7	73	2,325	5	60	875	38	1.18
8	9.5	87	1,900	5	40	163	36	1.38
9	10.4	86	3,125	5	60	1,412	33	1.36
10	13.2	97	2,775	4¾	43	720	32	3.54
11	9.5	83	1,900	5	40	355	32	1.00
12	16.8	83	7,000	5	83	4,428	30	1.00
13	11.3	86	2,800	4¾	50	1,305	27	1.34
14	13.6	90	2,750	5	40	907	27	1.29
15	13.2	83	2,640	5	40	2,210	6	0.81

employed as the objective criterion of pulmonary congestion and edema. As it happened, none of the ratios fell in the range 1.41-1.6%. When, for convenience, all animals falling under the criterion for "moderate" and "severe" pulmonary edema were grouped together, the data fell into three major groups as follows:

Group I = Lung Weight/Body Weight less than 1.20%:
No pulmonary edema (5 dogs)

Group II = Lung Weight/Body Weight 1.21-1.40%:
Questionable edema (6 dogs)

Group III = Lung Weight/Body Weight greater than 1.6%:
Moderate, or severe pulmonary edema (4 dogs)

As is to be expected, the more fluid administered and the more retained, the greater was the tendency toward the development of pulmonary edema, but these relationships were again inconstant, as is obvious from Table 1 where the data have been arranged in order of amount of fluid retained per kilogram of body weight. The trends are indicated in Table 2, and have been charted on the basis of a total infusion time of five hours in Figures 1-3. It is apparent that the animals with the heaviest lungs at necropsy (Group III) also had, on the average, the lowest urinary excretion. This does not imply, of course, that a large intake necessarily determines a low

TABLE 2. INTAKE, OUTPUT, AND RETENTION OF FLUIDS AND CHANGE IN BLOOD VOLUME

Group: L.W./B.W.%	*Fluid in cc./kg./hr.*	*Fluid out cc./kg./hr.*	*Fluid retained cc./kg./hr.*	*% Increase in blood volume*
<1.20	64.2	29.8	34.4	19
1.21 — 1.40	68.0	23.8	44.2	23
>1.60	85.8	21.2	64.6	39

NOTE: Each datum represents the mean of observations on at least three animals.

urinary output. As a rule, edema occurred if the rate of administration of the saline fluid was over 80 cc./kg./hour, and if the rate of retention exceeded 65 cc./kg./hour. The exceptions were in both directions. Thus, there was striking pulmonary edema in one dog (Table 1, #10), where the rate of administration had been only 43 cc./kg./hour and the retention rate 32 cc./kg./hour (total retention 2,592 cc. in a dog weighing 13.2 kg.). Within one-half hour after the beginning of the infusion his hematocrit had fallen from 44 to 34 (blood volume increased 29% above its initial level). One hour before death, when there was already clinically obvious edema, with abundant sanguineous froth in the tracheal tube, the hematocrit was 47, actually greater than it was initially. This signifies a loss of fluid from the blood at this time more rapid than was being replaced by the infusion. On the other hand, there was no evidence of significant pulmonary edema in another animal of the same weight (Table 1, Dog #2) with an input as large as 115 cc./kg./hour, that had received a total of 7,600 cc. of fluid in a five-hour period, during which interval he had retained 5,183 cc. of fluid (retention rate 78 cc./kg./hour). Urinary flow in this dog was relatively higher than the average for any group, 37 cc./kg./hour. It is remarkable

that at the end of the period of infusion this animal weighed almost 40% more than at the beginning. The hematocrit fell relatively little (from 37.3 to 31) during the infusion, indicating that the infusate had been largely transferred from the vascular compartment.

In all groups, changes in the hematocrit took place more rapidly at first, the greatest drop occurring in the first half hour after the beginning of the infusion, and continuing but very slowly thereafter. The greatest mean fall took place in the Group III animals, although in one dog (Table 1, #10), there was a late rise. Loss of fluid from the vessels was possibly abetted by anoxia as indicated by a large late fall in arterial oxygen saturation in this animal.

TABLE 3. CHANGES IN HEMATOCRIT AND CO CAPACITY DURING INFUSION OF FLUIDS

	Time after infusion began											
	0		*½ hr.*	*1 hr.*		*2 hrs.*		*3 hrs.*		*4 hrs.*		*5 hrs.*	
	*CO**	*Hct**	*Hct*	*CO*	*Hct*	*CO*	*Hct*	*CO*	*Hct*	*CO*	*Hct*	*CO*	*Hct*
I	15.7	40.0	34.7	14.7	35.5	13.7	33.2		31.7		29.7	12.7	33.6
II	16.2	39.1	34.9	15.3	33.4		32.5	14.1	33.0		31.6		
III	16.9	39.5		14.2	30.4		32.6		31.5	12.8	32.0		28.8

* Each datum represents the mean values obtained in at least three animals, in duplicate for each animal. All available data meeting the criteria are recorded.

The carbon monoxide capacity of the blood followed the same trend as the hematocrit (Table 3, and Figs. 1-3). It was apparent at post-mortem examination that most of the fluid was stored in the tissues, especially the peritoneal cavity, and to some extent in the subcutaneous tissues. There was relatively little fluid in the pleural or pericardial cavities.

In the figures, fluid input and retention are charted as straight lines. Inspection of the actual fractional output data reveals that after the first hour during which it was relatively low, the urine output remained at an almost constant increased level.

One striking finding in animals whose lung weight exceeded 1.6% of the body weight (the criterion for moderate or severe edema) was the steep rise of mean left atrial pressure. It reached levels in excess of 20 cm. of water after the infusion had been proceeding for 3½ hours, to attain a mean level of 27.5 cm. of water at the fifth hour. Even here there were some exceptions, for in one animal the mean atrial pressure at no time exceeded

17 cm. of water. In the presence of hemodilution as calculated from the hematocrit values, it may be presumed that even 20 cm. of water exceeded the oncotic pressure. Yeomans, Porter, and Swank[19] have demonstrated a fall in blood proteins in step with the degree of hemodilution resulting from the saline infusion.

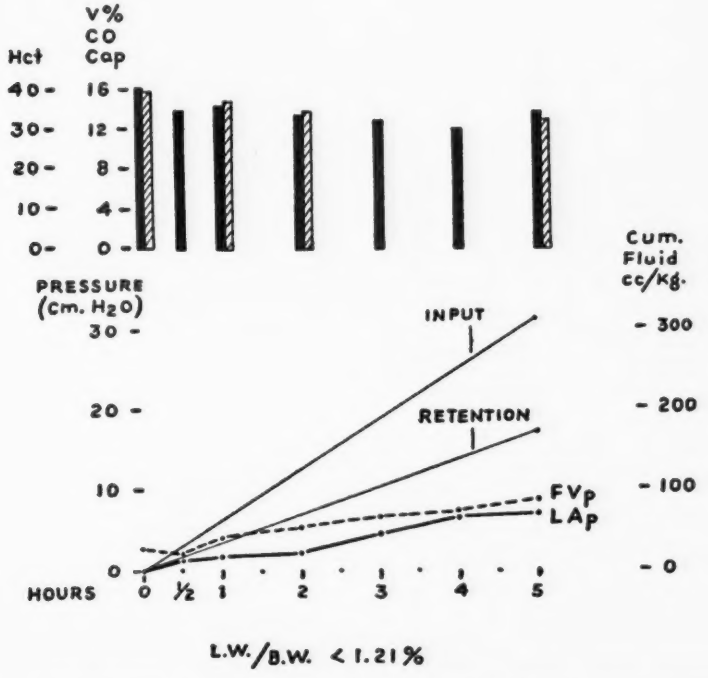


FIG. 1. Observations in five dogs without pulmonary edema. Abscissa represents duration of infusion, upon which all other observations are charted. In lower left of chart, input and retention of fluid are charted against scale at right, and femoral venous (FVp) and left atrial pressures (LAp) against scale at left. In upper half of chart, hematocrit and carbon monoxide capacity are indicated as black and cross-hatched columns, respectively.

The upward slope of the femoral venous pressure curve was less steep than that of the left atrial pressure and the absolute levels at the end of the five-hour period were much lower, yet, ascites, and in some animals subcutaneous edema, were striking even without evidence of pulmonary edema. This is indicative of the great importance of a fall in oncotic pressure in the peripheral capillaries, where the hydrostatic pressure is normally higher than in the pulmonary capillaries.

The pulmonary arterial pressure followed the trend of the left atrial pressure. There was in each instance a rise, but the slope of the upward curve was again less steep than that of the left atrial pressures. In Dog #10 (Table 1) the control pressures in the left atrium and pulmonary artery were respectively —2 and 24/10 cm. water, and at the end of five hours, when pulmonary edema was clinically obvious, the left atrial pres-

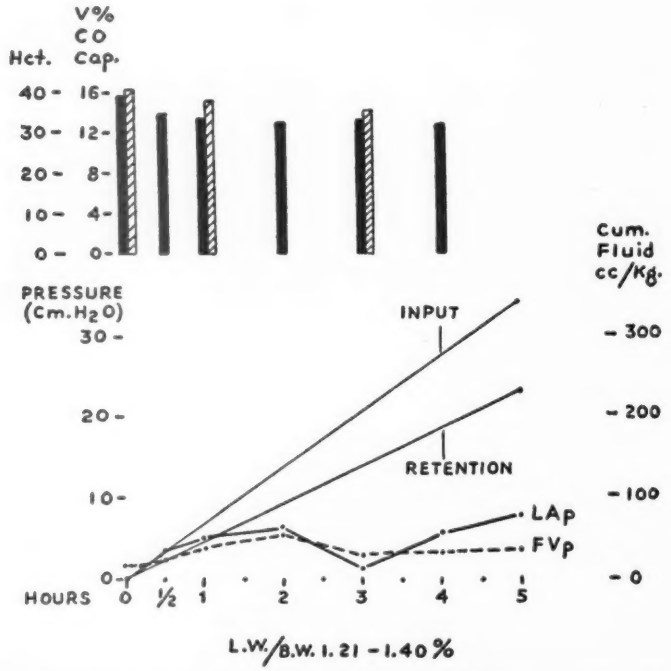


FIG. 2. Observations in six dogs with "questionable pulmonary edema (Group II)." Key as in Fig. 1. Except for greater input and retention of fluid, the data do not deviate markedly from those recorded in Fig. 1.

sure was 48 cm. of water and the pulmonary arterial pressure was 76/16 cm. of water (mean = 63 cm.).

Even in animals receiving the largest infusions, there was a drop in aortic diastolic pressure, sometimes in excess of 25 mm. of mercury. The systolic pressures usually changed only slightly. Pulse pressures generally were elevated. After the first hour, all pressures tended to remain relatively constant to the end of the experiment. The observations suggest dilatation of the systemic arteriolar bed, but may be explained also on the basis of decreased viscosity of the blood consequent to hemodilution.

DISCUSSION

As in previous observations[2, 9] on the effects of increased intracranial pressure, one highly important factor in the development of pulmonary edema appeared to be the maintenance of a pressure in the pulmonary capillaries in excess of 20 cm. of water, for an interval of time greater than

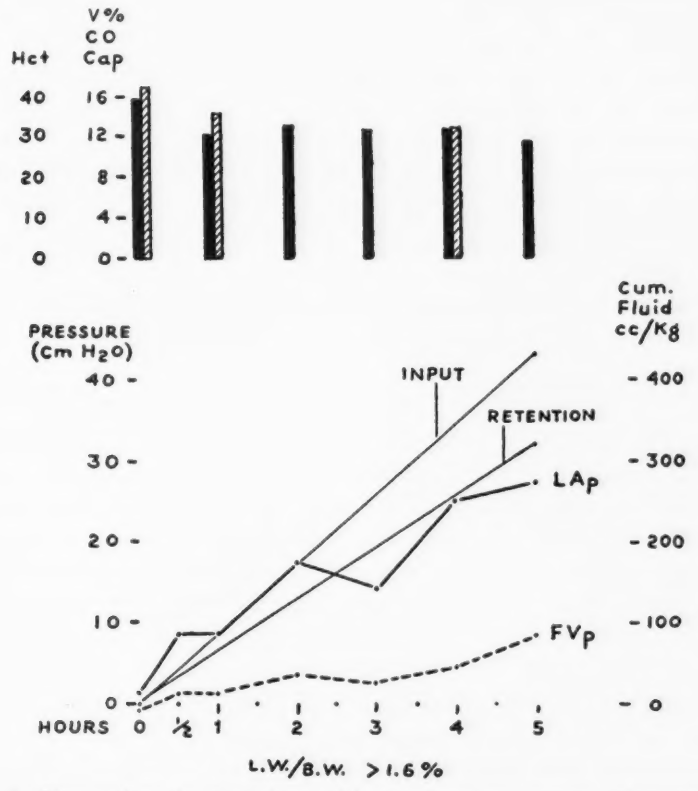


FIG. 3. Observations in four dogs with "moderate or severe pulmonary edema (Group III)." Key as in Fig. 1. The relatively sharp rise in the left atrial pressure is evident.

15 minutes. In the present experiments, hemodilution also played an important rôle.

The factors that contribute to the elevation of pulmonary capillary pressure are a high retention of fluid, especially in the intravascular compartment, associated not only with a high rate of infusion, but also with a low excretion rate. One of the most important questions is the mechanism responsible for the relatively low urinary excretion in some animals. No

ready answer is available from the data at hand. None of the animals in Group III could be considered in shock until the very termination of the experiment, with one possible exception. Even in this instance the aortic blood pressure had changed from 198/125 at the start to 140/70 at the fifth hour, possibly a "relative" shock level (Dog #1).

It may be asked whether the "pulmonary edema" in the animals of Group III is reversible. It was indeed necessary to sacrifice three of these animals at the end of the fifth hour, and only one (Dog #10) died spontaneously in pulmonary edema. The lung weight/body weight ratio in this animal was the highest of any (3.54%).

The presence of large quantities of ascitic fluid in some of the animals is of interest. Some animals with large amounts of ascitic fluid had normal lung weight/body weight ratios. Ascitic fluid was found in large quantities, in the absence of obvious subcutaneous edema or pleural or pericardial fluid. This is of interest since Yeomans and his co-workers[19] have demonstrated that portal venous pressure, like the pulmonary venous pressure, rises more steeply than the systemic venous pressure when large infusions are introduced intravenously.

The observed steeper rise of the pulmonary venous pressure in contrast with the systemic venous pressure confirms in a larger series under conditions of slower infusion, over longer intervals of time, and in animals with closed thorax, some observations of other workers and is indicative of a higher volume elasticity coefficient of the segment left atrium-pulmonary veins in comparison with the systemic venous bed, as suggested by Opdyke[13] and his group. When this limit of distensibility is approached, any increase in the burden of the heart such as would produce even a slight retention of blood in the left atrium, will result in a very steep rise in the pressure within this chamber and its venous tributaries, as Sarnoff[14–18] and his associates have emphasized. Thus, when the intracranial pressure is elevated at an appropriate rate, it is a conspiracy of an induced peripheral resistance and of factors tending to distend the atrium that disposes to pulmonary congestion and edema.[2, 9] This tendency will be augmented by any factor, such as an excess of infused fluids, tending to increase either of the contributing elements.

SUMMARY

When large quantities of saline fluids are administered to dogs over a five-hour period, pulmonary edema (as measured arbitrarily by the lung weight/body weight ratio) tends to develop in those animals that have the highest intake of fluid, lowest urinary output (concomitantly greatest reten-

tion), and highest ultimate blood volume, but many exceptions exist. The mechanisms responsible for these variations in excretion are not apparent from available data. One factor of significance in animals that develop pulmonary edema is the high pulmonary venous pressure that ultimately comes to exist at levels in excess of 20 cm. of water. The rise in pulmonary venous pressure is relatively rapid in comparison with that which occurs concomitantly in the systemic veins, confirming previous suggestions of a relative indistensibility of the pulmonary venous-left atrial segment. The rise in blood volume occurs quickly at first, but then tends to change only slightly despite the rapid infusion of large quantities of saline fluids. Thus the retained fluid finds its way into tissue spaces and especially into the peritoneal cavity, but only to a slight extent into the pleural cavities and pericardial sac.

REFERENCES

1 Altschule, M. D. and Gilligan, D. R.: The effects on the cardiovascular system of fluids administered intravenously in man. II. The dynamics of the circulation. J. Clin. Invest., 1938, *17*, 401-411.

2 Campbell, G. S., Haddy, F. J., Adams, W. L., and Visscher, M. B.: Circulatory changes and pulmonary lesions in dogs following increased intracranial pressure, and the effect of atropine upon such changes. Am. J. Physiol., 1949, *158*, 96-102.

3 Doyle, J. T., Wilson, J. S., Estes, E. H., and Warren, J. V.: The effect of intravenous infusions of physiologic saline solution on the pulmonary arterial and pulmonary capillary pressure in man. J. Clin. Invest., 1951, *30*, 345-352.

4 Drinker, C. K.: *Pulmonary edema and inflammation*. Cambridge, Mass., Harvard University Press, 1945.

5 Fegler, J. and Banister, J.: Congestive atelectasis in lungs of rabbits and other animals subjected to the action of low barometric pressure. Quart. J. Exp. Physiol., 1944-46, *33*, 291-309.

6 Gibbon, J. H., Jr. and Gibbon, M. H.: Experimental pulmonary edema following lobectomy and plasma infusion. Surgery, 1942, *12*, 694-704.

7 Gilligan, D. R., Altschule, M. D., and Volk, M. C.: The effect on the cardiovascular system of fluids administered intravenously in man. I. Studies of the amount and duration of changes in blood volume. J. Clin. Invest., 1938, *17*, 7-16.

8 Harrison, W. and Liebow, A. A.: Polyethylene plastic needle guides. Proc. Soc. Exp. Biol., N. Y., 1949, *70*, 421.

9 Harrison, W. and Liebow, A. A.: The effects of increased intracranial pressure on the pulmonary circulation in relation to pulmonary edema. Circulation, 1952, *5*, 824-832.

10 Jenkins, M. T., Jones, R. F., Wilson, B., and Moyer, C. A.: Congestive atelectasis. A complication of the intravenous infusion of fluids. Ann. Surg., 1950, *132*, 327-347.

11 Kawano, K.: A new fact and problem concerning the permeability of venules. (1). On the correlation between the emigration of leukocytes and permeability of dye stuffs. Acta. path. Japon., 1950, *1*, 48-55.

12 Luisada, A. A. and Sarnoff, S. J.: Paroxysmal pulmonary edema consequent to stimulation of cardiovascular receptors. I. Effects of intra-arterial and intravenous infusions. Am. Heart J., 1946, *31*, 270-280.

13 Opdyke, D. F., Duomarco, J., Dillon, W. H., Schreiber, H., Little, R. C., and Seely, R. D.: Study of simultaneous right and left atrial pressure pulses under normal and experimentally altered conditions. Am. J. Physiol., 1948, *154,* 258-272.

14 Sarnoff, S. J., Goodale, W. T., and Sarnoff, L. C.: Graded reduction of arterial pressure in man by means of a thiophanium derivative (Ro 2-2222). Preliminary observations on its effect in pulmonary edema. Circulation, 1952, *6,* 63-73.

15 Sarnoff, S. J. and Sarnoff, L. C.: Neurohemodynamics of pulmonary edema. 1. Autonomic influences on pulmonary vascular pressures and the acute pulmonary edema state. A preliminary report. Dis. Chest, 1952, *22,* 685-693.

16 Sarnoff, S. J. and Sarnoff, L. C.: Neurohemodynamics of pulmonary edema. II. The role of sympathetic pathways in the elevation of pulmonary and systemic vascular pressures following the intracisternal injection of fibrin. Circulation, 1952, *6,* 51-62.

17 Warthen, H. J.: Massive intravenous injections. An experimental study. Arch. Surg., 1935, *30,* 199-227.

18 Wiggers, C. J.: *Physiology in health and disease.* P. 695, 3d ed., Philadelphia, Lea & Febiger, 1939.

19 Yeomans, A., Porter, R. R., and Swank, R. L.: Observations on certain manifestations of circulatory congestion produced in dogs by rapid infusion. J. Clin. Invest., 1943, *22,* 33-45.

FRANCIS L. BLACK*
JOSEPH L. MELNICK

Section of Preventive Medicine, Yale University School of Medicine

THE SPECIFICITY OF THE COMPLEMENT FIXATION TEST IN POLIOMYELITIS**

Infection with poliomyelitis virus gives rise to complement-fixing (C-F) antibodies which may be detected with antigens prepared either from the brains of infected mice or tissue culture fluids, as shown with Type II by Casals *et al.*[1,2] and with all three types by Svedmyr *et al.*[9] These workers used sufficient antigen in each test to obtain maximum sensitivity. With this antigen concentration the presence of antibody could generally be detected within the first week after onset of the disease, but the sera usually gave similar C-F titers against antigens of the three types of poliomyelitis virus regardless of the type involved in the infection. It has now been found possible to obtain a more specific reaction in patients with the disease by a method in which not more than one conventional unit of antigen is used per test. In order to detect fixation with this low level of antigen, dilutions of serum were titrated at several complement concentrations by the plate method of Fulton and Dumbell.[3]

METHODS AND MATERIALS

The general technique of the plate C-F method as used in this laboratory has been described previously.[4] This method has already been used in poliomyelitis work by Svedmyr *et al.*,[9] and by Le Bouvier.[5] Instead of glass capillaries, the dropping pipettes used in this study consisted of No. 19 needles, with the bevelled tip removed,[9] fitted to "oiling tubes."† The unit in which *complement* was used was the greatest amount of freshly reconstituted, lyophilized guinea pig serum in a 20-cm. drop that caused no visible hemolysis in the test system. This "minimal unit" is approximately one-third of a conventional 100% lytic unit. Serum and antigen titrations were carried out against two, three, and four of these units and the controls were tested against one, two, and three units.

---

* Fellow of the Rockefeller Foundation.

** Aided by a grant from the National Foundation for Infantile Paralysis.

† Macalaster Bicknell Co. Inc., New Haven, Conn.

Received for publication April 6, 1954.

Antigens were prepared from virus grown to high titer (10^6 to 10^8 TC_{50} doses per ml.) in bottle-cultures of kidney tissue[6] and of HeLa cells.[5]* The Brunhilde and W-S (Type I), Y-SK (Type II), and Leon (Type III) strains were found to be satisfactory for the preparation of antigens. Fluids

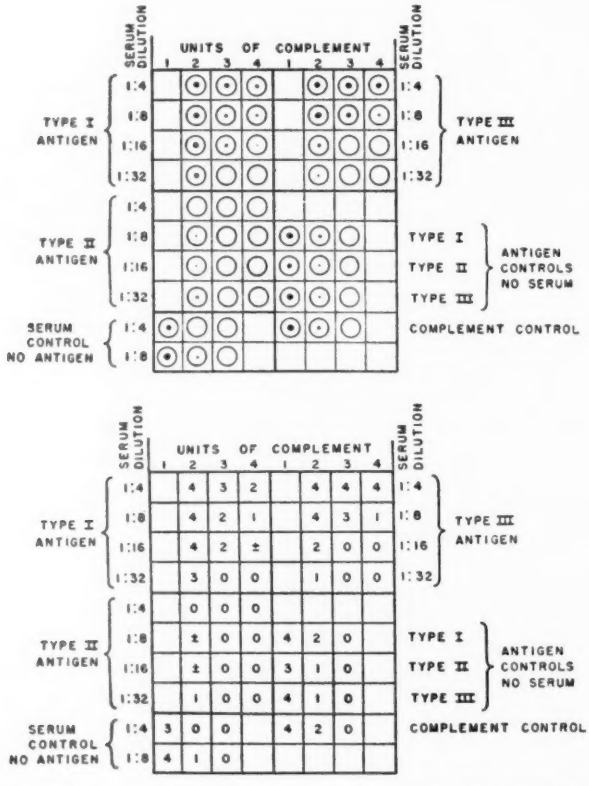


FIG. 1. Appearance of a typical plate at time of reading, and corresponding fixation scores.

from the tissue cultures were harvested when most of the cells had lysed (after 16 to 64 hours exposure to virus depending on the inoculum). These fluids were lightly centrifuged to remove cellular debris and were generally used without further treatment. The amount of antigen required to fix one minimal unit of complement in excess of the greatest amount fixed by any of the controls was termed one minimal unit. This is between one-half and

* Some of the HeLa bottle-cultures were obtained ready for use from Microbiological Associates, Bethesda, Maryland.

one-fourth of a conventional unit. Fluids containing less than two units per 20 cm. drop could be concentrated by centrifugation at 40,000 R.P.M. (about 106,000 x gravity) for two hours, and resuspension of the pellet in a small volume of diluent, but this was usually not necessary. Occasional lots were found anticomplementary in which case they could often be made serviceable by heating at 56° C. for 30 minutes.[9] Heating the antigens had the added advantage of rendering them non-infectious.

Sera were titrated with two minimal units of antigen per test drop against three concentrations of complement. The titer of a serum was taken as the highest dilution to fix more than one minimal unit of complement in excess of the amount fixed by either serum or antigen control. (For example in the Type I titration illustrated in Figure 1, the greater amount of fixation is in the antigen control. The highest serum dilution to give fixation of more than one unit in excess of this control is 1 : 16 and the serum titer is 16.) As well as the titer, it was possible to determine from such a two-dimensional titration, the total amount of complement fixed. This character of the serum, its *avidity* for complement, is partially independent of the titer. An expression of the avidity dependent on both the titer of the serum and the amount of complement fixed was calculated as follows: (a) the score for the degree of fixation was summed for all serum dilutions up to the titration endpoint, and from this was subtracted (b) the number of dilutions tested up to the endpoint, multiplied by the sum of the fixation score for the control showing the greater degree of fixation. Serum scores, (a) above, were calculated for the titrations with two, three, and four units of complement and the control scores (b) for one, two, and three units and thus the avidity was positive only when more than one unit of complement was fixed. (In the Type I example again, the value for (a) is 22, and for (b) the subtrahend, based on the antigen control, is 6 x 3 or 18. The avidity score is therefore four. The avidity for the Type III titration in Figure 1 is ten, although the titer is only eight.) With the human sera the avidity was found more reproducible and showed fewer erratic variations between serial serum samples than the simple serum titer.

RESULTS

Standard monkey sera. Monkey sera prepared and kindly supplied by Wenner *et al.*[10] were titrated in two-fold dilutions against various antigens at several concentrations. The results are given in Table 1. The titers obtained with the higher antigen concentrations were comparable to those found in the laboratories of Wenner and of Enders, and the slight crossing between the Type II serum and Type I antigen was confirmed.[10] The titers

found when approximately two minimal units of antigens were used were somewhat lower. Approximately two units were used in the studies on human sera reported below.

TABLE 1. TITER OF STANDARD POLIOMYELITIS SERA AGAINST DIFFERENT CONCENTRATIONS OF ANTIGENS

Antigens			*Titer of standard monkey sera*		
Virus strain	*Cell culture used*	*No. of minimal units*	*I*	*II*	*III*
Brun.	Kidney	3.5	>64	<4	<4
WS	HeLa	3.0	32	<2	<2
Brun.	Kidney	2.7	32	<2	<2
WS	HeLa	2.1	32	<4	<4
Brun.	Kidney	2.0	16	<2	<2
WS	HeLa	1.5	32	<4	<4
Brun.	Kidney	1.3	16	<2	<2
YSK	Kidney	3.5	4	>64	<4
YSK	HeLa	3.0	2	32	<2
YSK	Kidney	2.7	tr*	16	<2
YSK	HeLa	2.1	tr	16	<4
YSK	Kidney	2.0	tr	8	<2
YSK	HeLa	1.5	<8	<8	<4
YSK	Kidney	1.3	tr	4	<2
Leon	Kidney	4.0	<4	<4	>64
Leon	Kidney	3.3	2	<2	64
Leon	Kidney	2.5	<4	<4	32
Leon	HeLa	2.0	....	<2	16
Leon	Kidney	1.7	<8	<4	16
Leon	HeLa	1.4	....	<2	16
Leon	HeLa	1.0	....	<2	tr

* tr indicates trace (less than 2).

*Sera from poliomyelitis patients.** Acute and convalescent sera from 49 patients diagnosed as poliomyelitic were tested for C-F antibodies against the three types of antigen. These sera had previously been tested for neu-

* Material obtained from patients in the Lock Haven, Pennsylvania, epidemic of 1952. Detailed virological study in which Drs. Manuel Ramos Alverez and Anthony J. Girardi actively participated will be presented in a separate paper.

tralizing antibody, and stools from most of the patients had been tested for virus.[11] Both Type I and II viruses, but no Type III virus had been isolated from the stools. Some examples of the results are given in Table 2, and the

TABLE 2. EXAMPLES OF DATA OBTAINED ON LOCK HAVEN CASES

Patient no.	*Type of virus isolated*	*Clinical evidence of paralysis*	*Date of serum**	*Complement fixation*						*Neutralization titers*		
				Titers			*Avidity*					
				I	*II*	*III*	*I*	*II*	*III*	*I*	*II*	*III*
83	I	+	7	0	0	16	0	0	2	160+	20	0
			49	32	0	4	15	0	1	160+	40	0
			70	32	0	0	19	0	0	160+	160+	0
93	II	+	3	0	0	0	0	0	0	0	10	0
			33	0	32	0	0	15	0	0	100+	0
			44	0	32	0	0	31	0	0	100+	0
8	Neg.	—	3	0	0	0	0	0	0	100+	0	0
			21	4	0	0	1	0	0	100+	0	0
			50	16	0	0	7	0	0	100+	0	0

* Days after onset.
0 indicates less than 4.
32 indicates 32 or greater.

TABLE 3. SUMMARY OF RESPONSES IN C-F ANTIBODIES IN POLIOMYELITIS PATIENTS

Type of virus isolated	I	I	II	II	0	0
Paralysis	+	—	+	—	+	—
Antibody responses	No. of patients in each category					
Against one type	15	12	4	1	0	4
Against two types	3	0	1	0	0	0
Against three types	0	0	0	0	0	0
Against no type	0	1	1	2	2	3

types of response found in all the sera are classified in Table 3 by type of disease. All patients from whom virus was isolated had homotypic neutralizing antibodies in their serum and frequently this was already at high titer at the time of the first serum collection (0-14 days after onset), e.g. No. 83. In contrast homotypic C-F antibodies were rarely detectable in the first

serum sample by the method which has been described. Of the 40 patients studied from whom virus was isolated only three (2 Type I, 1 Type II) had demonstrable homotypic C-F antibodies at the time the first specimen was collected, 0-14 days after onset. In six cases the antibodies first became demonstrable in the third specimen more than 30 days after onset. More heterotypic responses were found in the neutralizing than in the C-F antibodies. There was no significant difference in the response of nonparalytic and paralytic patients regardless of whether infected with Type I or II viruses.

On the basis of the findings of Casals, Svedmyr, and their co-workers,[2,9] it was expected that the highest titers of C-F antibody would be found in the sera collected in the first few weeks after onset. However, in the present study homotypic C-F antibody was rarely found before seven days after the onset of the disease and often increased after the third week. Because of this it was possible to demonstrate an increase in titer more frequently than might otherwise have been the case. On the other hand it was not possible with this method to demonstrate such a rise until some weeks after the individual had become sick.

The above investigators had found that many persons infected with Type I poliomyelitis developed C-F antibodies against Type II antigen. Yet in our study only one individual infected with Type I virus showed a Type II C-F response, nor did the reverse heterologous response, Type I C-F antibodies in Type II infection, occur more frequently. These differences between the results of our own studies and those from the two other laboratories appear to be due to the smaller amounts of antigen used in the present work. Casals used 8 to 16 units of antigen in each test and Svedmyr an approximately equivalent amount, whereas the amount of antigen used in our study was not more than one conventional unit. Serum samples from poliomyelitis patients have been exchanged with Dr. John F. Enders and the results obtained by the method described in the present report proved more specific than those obtained with concentrated antigens.[9]

Sera from children inoculated with formalinized Type I virus. The details of this experiment[11] will be reported elsewhere; here we merely wish to call attention to the application of the complement fixation test to this type of investigation. Thirty apparently healthy children aged 12 to 17 were tested for C-F antibodies at the time of inoculation and 3 and 20 weeks after inoculation with a formalin-inactivated Type I virus preparation, similar to that used by Salk.[7] At the time of inoculation Type I C-F antibodies were not present at a detectable level in any individual, and Types II and III antibodies were each present in only one individual. The changes found are

given in Tables 4 and 5. Where antibodies appeared, they reached a peak titer of eight or greater in all but three instances. The responses in this group occurred, with but three exceptions, only against those types with which there had been previous experience as indicated by the neutralization tests that had been made before inoculation. Two of the three exceptions

TABLE 4. CHANGES IN C-F ANTIBODIES IN CHILDREN AFTER INOCULATION WITH TYPE I FORMALIN-INACTIVATED VIRUS

Type of antibodies	*Neutralizing antibodies prior to inoculation*	*Number without C-F antibodies pre-inoc.*	*C-F antibody response*	
			positive	*negative*
I	—	10	1	9
	+	20	8	12
II	—	15	1	14
	+	15	9	6
III	—	13	1	12
	+	13	7	6

TABLE 5. CHANGES IN TYPES I AND II C-F ANTIBODIES IN CHILDREN AFTER INOCULATION WITH TYPE I FORMALIZED VACCINE

Result of neutralization tests made prior to inoc.		*Total number of cases*	*I Increased II Negative**	*II Increased I Negative*	*I and II Increased*	*Both negative*
Type I	*Type II*					
—	—	7	0	0	1	6
+	—	8	4	0	0	4
—	+	3	0	2	0	1
+	+	12	1	4	3	4
Total		30	5	6	4	15

* Negative indicates serum titer of less than 2.

occurred in one individual where Types I and II antibodies appeared in the 20-week sample. The findings in healthy children inoculated with formalin inactivated virus were in marked contrast to those in poliomyelitic patients reported above. In the latter the response was usually only homotypic with the type of virus causing the infection. In the "vaccine" study where all the responses were caused by Type I virus material the

antibodies to the three types of virus responded similarly. The Type III response was somewhat weaker than the Types I and II reaching lower maximal titers (median for Type III was 8; for Types I and II, 32) and falling off more rapidly. Table 5 shows that the presence of Type I neutralizing antibody had no significant effect on the probability of a Type II C-F response and vice versa. The Type III C-F response was also independent of Types I and II neutralizing antibodies, and Types I and II C-F responses of Type III neutralizing antibodies, but to have included these combinations would have overburdened the table. The reason for the contrasting nonspecificity of the response in the group of children in whom antibodies were stimulated artificially as compared to the response in the natural infections, has not been found as yet.

SUMMARY

A complement fixation test for poliomyelitis which uses minimal quantities of complement and antigen has been described and its application noted in two phases of poliomyelitis investigations: serodiagnosis of infection and antibody response to "vaccination": (i) Results of tests for C-F antibodies for the three types of virus are reported on specimens obtained from 49 poliomyelitis patients. The results show a homotypic C-F titer rise in 90% of the patients from whom virus was isolated, and a heterotypic rise in 10%. (ii) Sera from 30 children before and after inoculation with formalin-inactivated Type I virus were tested for C-F antibodies. Of these, 60% showed a post-inoculation increase in Type I, II, or III antibodies. Some showed C-F responses to antibodies of more than one type. Nearly all of the increases occurred in individuals who possessed pre-inoculation neutralizing antibodies.

REFERENCES

1 Casals, J., Olitsky, P. K., and Anslow, R. O.: A specific complement fixation test for infection with poliomyelitis virus. J. Exp. M., 1951, *94*, 123.

2 Casals, J., Olitsky, P. K., and Sabin, A. B.: Development, persistence and significance of Type 2 poliomyelitis complement fixing antibody in man. J. Exp. M., 1952, *96*, 35.

3 Fulton, F. and Dumbell, K. R.: The serological comparison of strains of influenza virus. J. Gen. Microb., Lond., 1949, *3*, 97.

4 Kraft, L. M. and Melnick, J. L.: Immunological reactions of the Coxsackie viruses. J. Exp. M., 1950, *92*, 483.

5 Le Bouvier, G. L.: Observations on the poliomyelitis complement fixation test. Brit. J. Exp. Path., 1953, *36*, 300.

6 Morann, G. L. and Melnick, J. L.: Poliomyelitis virus in tissue culture VI. Use of kidney epithelium grown on glass. Proc. Soc. Exp. Biol., N. Y., 1953, *84*, 558.

7 Salk, J. E.: Studies in human subjects on active immunization against poliomyelitis. J. Am. M. Ass., 1953, *151*, 1081.

8 Scherer, W. F., Syverton, J. T., and Gey, G. O.: Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (Strain HeLa) derived from an epidermoid carcinoma of the cervix. J. Exp. M., 1953, *97*, 695.

9 Svedmyr, O., Enders, J. F., and Holloway, A.: Complement fixation with the three types of poliomyelitis viruses propagated in tissue culture. Am. J. Hyg., 1953, *57*, 60.

10 Wenner, H. A., Miller, C. A., Kamitsuka, P., and Wilson, J. C.: Preparation and standardization of antiserums prepared with the three known types of poliomyelitis viruses. Am. J. Hyg., 1954, *59*, 221.

11 Yale Poliomyelitis Study Unit, unpublished work.

ROBERT H. DRACHMAN*
JAY TEPPERMAN

Department of Pharmacology, State University of New York, Upstate Medical Center, Syracuse, New York

AUROTHIOGLUCOSE OBESITY IN THE MOUSE

In 1816 William Wadd[31] remarked that "corpulency" occurred very frequently after the use of mercury in the treatment of syphilis, and he stated that preparations containing this metal ". . . might almost be recommended as specific agents for bringing about the accumulation of fat." The next observation linking a heavy metal with obesity was made by Brecher and Waxler,[3] who reported in 1949 that striking obesity often occurred in mice injected with a large dose of aurothioglucose (ATG), a compound that had been used in 1927 by Lande[16] for the treatment of arthritis.

Brecher and Waxler[33] demonstrated that a single injection of ATG can produce massive and persistent obesity in mice, but that it is necessary to use a dose of the compound which approximates its $L.D._{50}$ (lethal dose) to secure this effect. A variable number of the survivors of such a dose (usually about a third) exhibit obesity of varying degree. It was clearly shown that the obesity is related to a marked increase in spontaneous food intake, and that there is a close correlation between increased food intake and the extent of the obesity.

Our present knowledge of this chemically-induced form of obesity can be summarized as follows: (i) Different strains of mice appear to be variably susceptible to the toxic effect of ATG.[1] (ii) The syndrome has been seen only in the mouse, although Waxler and Brecher[1, 2] have attempted to reproduce it in guinea pigs, rats, rabbits, and puppies. (iii) Obese mice have no apparent difficulty in mobilizing their depot fat, and, after periods of starvation, they overeat and become obese again.[33] (iv) Histological study of tissues from obese mice has revealed no lesions in the central nervous system or elsewhere which might contribute to an understanding of increased food intake.[3, 24] (v) A thioglucose compound without gold did not produce obesity in mice, while gold thiomalate injections resulted in only slightly excessive weight gain.[1, 2]

The present report is concerned with two aspects of the problem. First, studies concerning the mechanism of production of obesity by aurothio-

* This study was made during tenure of a State University of New York Predoctoral Fellowship.

Received for publication January 26, 1954.

glucose will be described; and second, certain experiments which are related to possible adaptive responses to chronic overnutrition will be presented. This bipolar approach to the problem may be justifiable on two grounds: a better understanding of the mechanism of induction of obesity by aurothioglucose will yield interesting information about the operation of the hunger-satiety apparatus of normal animals. Secondly, a study of the metabolic impact of overnutrition on animals may provide a basis for a clearer comprehension of the patho-physiology of a number of "degenerative" diseases.[9]

MATERIALS AND METHODS

Approximately 1,500 female albino mice (Bio Lab Breeding Institute & Albino Farms) were used in this study. They were maintained in groups at a temperature of about 26° C., and were permitted free access to water and to a diet of Purina Dog Chow unless otherwise noted.

Aurothioglucose ("Solganal," supplied generously by Dr. Norman Heminway of the Schering Corporation) was used either as a sesame oil suspension containing 100 mgs. per ml. or (in later experiments) as a freshly prepared aqueous solution of a water-soluble powder. Injections were made either intraperitoneally or intramuscularly as noted.

Mouse tail blood samples (0.05 ml.) were analyzed for glucose content by the Nelson modification[22] of the Somogyi method. Liver fat content of 500 mg. samples was estimated by a modification of a method suggested by Payne.[25] The solvent was Bloor's reagent, and every precaution was taken to prevent evaporation of the solvent during filtration. Nitrogen was estimated on 50 mg. liver samples by a modification of the method of Koch and McMeekin.[15] Serum cholesterol determinations were performed according to the method of Sperry and Schoenheimer.[26]

The Haldane open circuit method[12, 7] was used for estimation of oxygen consumption of intact mice in the fed state. Oxygen consumption of liver slices was measured in a standard Barcroft-Warburg apparatus in phosphate buffer at pH 7.4, with oxygen as the gas phase.

Endocrine organs were weighed on a Roller Smith Torsion Balance, and tissues to be examined histologically were fixed in 10% formalin.

In all studies of obesity incidence reported herein a weight of 45 grams was arbitrarily selected as the minimum weight indicating the presence of obesity. The mean weights of different groups of uninjected controls varied between 27 and 33, and it was only extremely rarely that a control animal attained a weight of 40 grams. Most of the obese mice used in the experiments described in Part II weighed 55 grams or more. Mice which attained a weight of 80 grams or more were seen with some regularity. The maximum weight seen in this laboratory was 96 grams.

A purified pituitary growth hormone preparation was kindly supplied by Mr. Irby Bunding of the Armour Laboratories (Lot No. R491024).

Probabilities were estimated according to student's test of "t."[10]

EXPERIMENTAL DESIGN AND RESULTS

PART I. THE INDUCTION OF OBESITY BY AN INJECTION OF AUROTHIOGLUCOSE

1. *The effect of fasting on toxicity and obesity incidence due to aurothioglucose.*

In accordance with a suggestion by Brecher,[2] one mg. of aurothioglucose in oil per gram of body weight was injected intramuscularly into fed mice and into mice fasted for 24 hours. Food was withheld for an additional 22 hours from the fasted group, while the control group was permitted free access to food. The results are shown in Table 1. There is an indication that starvation increased both the survival rate and the incidence of obesity. In addition, a larger number of mice in the fasted groups attained extremely

TABLE 1. THE EFFECT OF FASTING ON TOXICITY AND OBESITY INCIDENCE FOLLOWING A SINGLE INJECTION OF AUROTHIOGLUCOSE IN SESAME OIL

Nutritional state	*Dose of ATG mgs./gm.*	*No. mice injected*	*Per cent survivors*	*Per cent obese of survivors*
Fasted	1.0	200	23	53
Fed	1.0	110	11	33

heavy weights. For example, the maximum weight observed in the fasted group was 91 grams, with many mice reaching 80 grams or more. The maximum weight observed in the fed group was 75 grams. The mean weight of sesame oil-injected controls in this series was 28 grams.

2. *Aurothioglucose toxicity in carbohydrate diet and fat diet-adapted mice.*

The increased rate of survival in the fasted group in Experiment 1 suggested the possibility that some metabolic concomitant of starvation exerts a protective effect against ATG. Since starvation and fat-feeding have many metabolic similarities, it was decided to test the effect of fat-feeding on ATG toxicity.

Accordingly, synthetic diets were prepared in which isocaloric quantities of lard and a glucose-sucrose mixture were added respectively to a basal amount of protein, (21 per cent of the total calories) salts, and vitamins. Groups of mice, which weighed from 11-16 grams, were fed these diets for a period of six weeks. At the end of that time they were injected intraperitoneally with 0.8 mgs. of ATG per gram of body weight. A smaller

group of chow-fed controls was fasted for 24 hours and injected at the same time. The results of this experiment are given in Table 2.

It will be seen that the mean body weight of the fat-fed mice at the time of injection was distinctly higher than that of the chow diet group, and thus, the mice in the former group received a slightly larger absolute dose of ATG than did the controls. The survival rate of the fasted chow-fed mice and the fed carbohydrate-fed ones was not strikingly different in this experiment, while the survival rate of the fat-fed mice was so much lower than that of the other two groups that the result could hardly have been due to a

TABLE 2. AUROTHIOGLUCOSE TOXICITY IN CARBOHYDRATE DIET AND FAT DIET-ADAPTED MICE

Diet group	*ATG* mgs./gm.*	*No. of mice injected*	*Mean body wt. gms. Injection time*	*Per cent survival*
Fat	0.8	137	28	17
Carbohydrate	0.8	110	25	40
Chow†	0.8	50	23	50

* Aqueous, i.p.

† Fasted 24 hours before and 22 hours after injection.

12 per cent increase in absolute dose. This result clearly differentiates fat-feeding from starvation as far as the susceptibility to ATG intoxication is concerned.

3. *The effect of treatment with Dimercatopropanol (BAL) on toxicity and obesity incidence due to ATG*

Many observations of the sort reported in experiments 1 and 2 led to an inquiry into whether or not the lethality of ATG could be shown to be separable from its obesity-producing potency. Apparently, this was accomplished to a certain extent in the starvation experiment, in which it was found that more mice survived and more of the survivors became obese. The following question was asked: if mice could be protected from dying a heavy metal death, would obesity-incidence be unaffected, and would it, therefore, be possible to increase the yield of obese mice per 100 injected?

In an attempt to answer this question mice were injected intramuscularly with 1 mg./gm. of ATG and BAL according to three different schedules.

Survival and obesity incidence were observed for a period of four months. The results are shown in Figure 1. It is obvious that, as protection against the lethal properties of the compound became more pronounced, the incidence of obesity among the survivors markedly diminished.

4. *The effectiveness of ATG at two widely different blood glucose levels*

The experiments with BAL suggested the possibility that the gold portion of the ATG molecule is essential to its capacity to produce obesity. The thioglucose moiety is closely related to glucose, and may contribute substantially to the effectiveness of the compound as an obesity-producing agent.

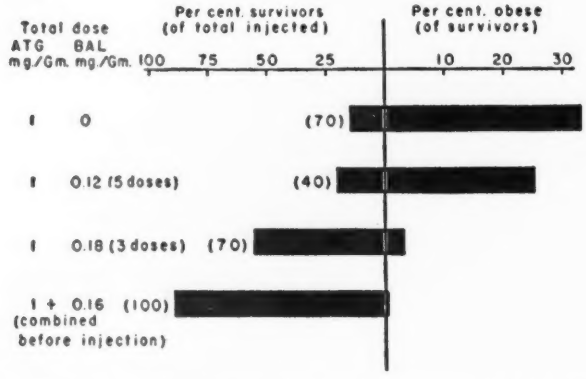


FIG. 1. The effect of treatment with dimercaptopropanol (BAL) on toxicity and obesity incidence after aurothioglucose injection.

Recently, Mayer and his associates[18, 19] have amplified earlier ideas into an elaborate hypothesis which they call the "glucostatic theory of food intake regulation." Some features of this hypothesis were used in designing the following experiment: there are glucoreceptors in the body which signal satiety to integrating centers as the concentration of glucose (or some metabolic derivative of glucose) in the body fluids and, subsequently, in the receptor cells themselves rises. The function of these cells can be permanently impaired by a single injection of aurothioglucose. The similarity in structure between glucose and ATG suggests the possibility of a competitive relationship between the two substances for a protein surface in the hypothetical receptor cells. Therefore, it was suggested that a very large excess of glucose in the body might serve to protect the hypothetical receptors from combination with ATG.

Accordingly, two similar groups of mice were fasted for 24 hours. The mice in Group A were injected intraperitoneally with 60 mgs. of glucose in 0.3 cc. of 0.9 per cent saline. Fifteen minutes later tail blood samples were obtained from 25 representative mice and all were immediately injected with 0.7 mg./gm. of ATG (aqueous) intraperitoneally. Tail blood samples were obtained from 25 mice in group B and all of them were then injected with ATG as in the case of group A. Food was returned to both groups and survival and obesity incidence were observed over a four-month period. The results of the experiment are seen in Figure 2. Instead of protecting the

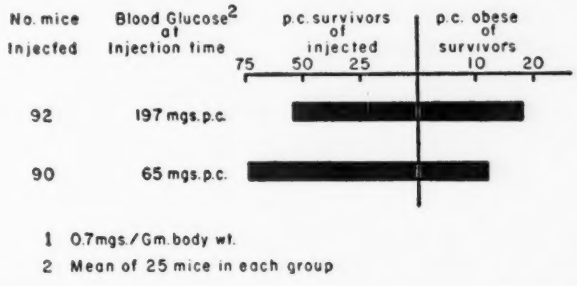


FIG. 2. The effect of antecedent glucose injection on toxicity of aurothioglucose[1] and on obesity incidence.

mice from ATG intoxication it is clear that, as a consequence of the glucose injection, the mice in the hyperglycemia group showed an *enhanced* susceptibility to the compound. Furthermore, there was a tendency toward an *increased* incidence of obesity among the survivors.

PART II. SOME EFFECTS OF LONG-ESTABLISHED ATG OBESITY ON THE MOUSE

1. *Carbohydrate metabolism*

In view of the well-recognized relationship between obesity and impairment of carbohydrate tolerance in the human[23] and in other forms of experimental obesity[6, 20] glucose tolerance tests were performed on a group of representative obese mice and on appropriate controls in the fed state. Glucose was injected intraperitoneally in a total volume of 0.3 to 0.4 ml. of solution. The control mice were given a dose of 1 mg. of glucose per gram of body weight, while the fat mice were all given 42 mgs. of glucose, which represented about a 40 per cent increase over that given as the mean control dose. Tail blood samples were obtained at 15, 45, and 90 minutes after glucose injection. The tests were repeated on each mouse after an interval

of four to eight weeks, and the means for each animal were used for Table 3, which shows the results of the experiment.

In spite of the fact that the obese mice weighed approximately three times as much as did the controls, and although they had been markedly obese for at least two months, their glucose tolerance curves did not differ much from those of the controls. The only noteworthy result of this experiment is the fact that the curves of the obese mice varied over a much wider range than did the control curves. It is possible that the extremely flat curves (1,587 area units) and the elevated ones (3,485 area units) represent enhanced and impaired glucose tolerance respectively. It should be noted that these mice were studied a maximum of five months after ATG injection.

TABLE 3. GLUCOSE TOLERANCE OF ATG OBESE MICE AND CONTROLS

	No. of mice	*No. of observations*	*Glucose tolerance area**	
			Mean	*Range*
Controls	5	10	2,446	2,070-2,975
Obese	5	10	2,583	1,587-3,485

* Arbitrary area units (inches ^{2}x100).

A parenthetical remark by Mayer[21] suggested the possibility that ATG obese mice might be hypersensitive to the hyperglycemic effect of certain growth hormone preparations. Accordingly, ten obese mice (50-79 gms.) and eight controls (30-38 gms.) were injected with four mgs. of Growth Hormone (Armour, Wilhelmi type). Ten other obese mice and eight suitable controls were similarly injected with crystalline bovine plasma albumin. Tail blood samples taken before and 24 hours after injection showed no discernible effect of either the Growth Hormone or the albumin on the blood glucose.

2. *Fat metabolism*

Previous studies of hypothalamic obese rats[5] showed striking increases in the fat content of the liver and in serum cholesterol and serum fatty acids over the levels found in control animals. It was of interest, therefore, to study these parameters in ATG obesity.

A highly significant increase in fat content over control values was found in the livers of ten obese mice. Similarly, there was a statistically significant increase in serum cholesterol in the obese mice (see Fig. 3).

3. *Studies on the so-called "hypermetabolism of obesity"*

There has been some uncertainty about the significance of measurements of oxygen consumption in obese animals. For example, it has been suggested that obese humans show hypermetabolism as compared with people of "ideal weight (see Evans[28]).

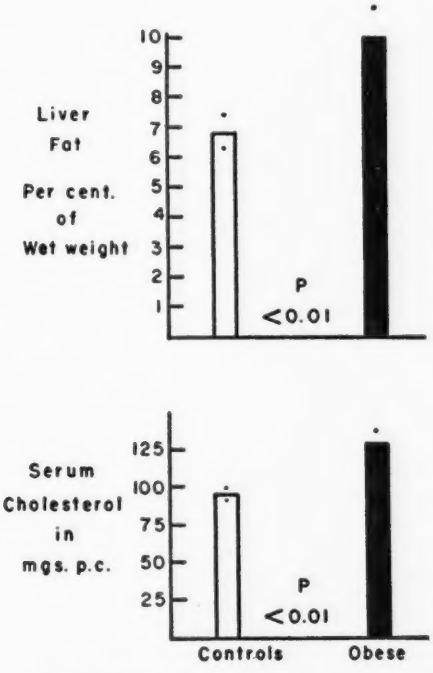


FIG. 3. Liver fat and serum cholesterol concentrations in aurothioglucose mice and controls.

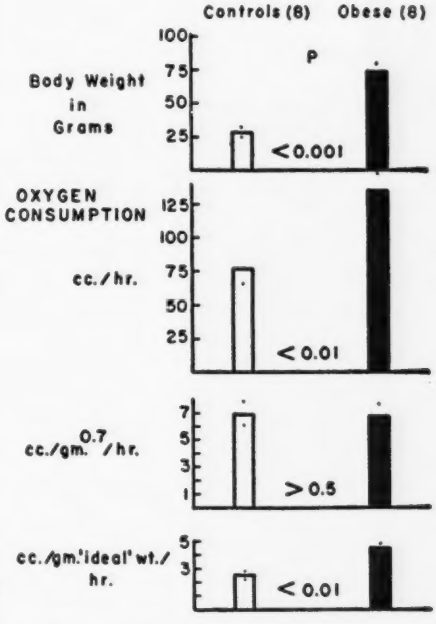


FIG. 4. The oxygen consumption of aurothioglucose mice and controls.

The nature of the supposed "hypermetabolism" of obesity has never been clearly elucidated. It was planned, therefore, to study the problem in ATG obese mice in an effort to obtain an answer to the following question: Does the high absolute oxygen consumption of the chronically overnourished animal signify an increase in rate of metabolism of an unchanged mass of contributory tissue, or is marked obesity due to chronic overnutrition invariably accompanied by an increase in the "active metabolic mass" of the animal?

Eight obese mice (mean weight 73.4 gms.) and eight controls (29.9 gms.) were selected for this part of the study. The total oxygen consump-

tion was estimated at least twice for each mouse, and the means for each animal were used for the calculations shown in Figure 4. It will be seen that there is a very marked increase in absolute oxygen consumption in the obese group. When the oxygen consumption is recalculated on the basis of a power function of the body weight which approximates surface area,[7] there is no difference between the groups. Recalculation on the basis of "ideal" weight reveals a significant difference between the groups.

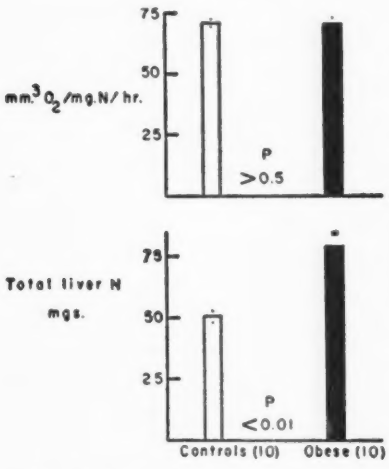


FIG. 5. The oxygen consumption of surviving liver slices of aurothioglucose mice and controls shown with total hepatic nitrogen values for the two groups of mice.

The nature of this difference was clearly demonstrated by the next experiment. Animals similar to those described above were sacrificed and the oxygen consumption of their surviving liver slices was measured in a Barcroft-Warburg apparatus. Since it had been determined that the obese mice showed fatty infiltration of the liver, the results were expressed not as Q_{O_2} but as mm^3O_2 consumed per mg. nitrogen per hour. On this basis, liver slices obtained from fat mice and those obtained from controls were quite indistinguishable from one another (see Fig. 5).

The absolute increase in oxygen consumption of the fat mice, then, can be attributed to the fact that, probably as a consequence of chronic overnutrition, they have acquired an increased "active metabolic mass," a true splanchnomegaly. This is evident not only in the liver nitrogen figures in Figure 5 but also in the section to follow.

4. *Autopsy data on ATG obese mice and controls*

At the time of sacrifice (five to seven months after injection) most of the obese mice appeared to be in good health. They did not show skin lesions on the legs or loss of hair such as are sometimes seen in old, hypothalmic obese rats.[39] Nose-to-anus and tail length measurements revealed no difference between the obese mice and their controls.

When the abdominal cavity was opened, massive deposits of fat were found in the subcutaneous tissue and in the mesentery (see Fig. 6). There was a clearly evident splanchnomegaly; all of the abdominal viscera in the

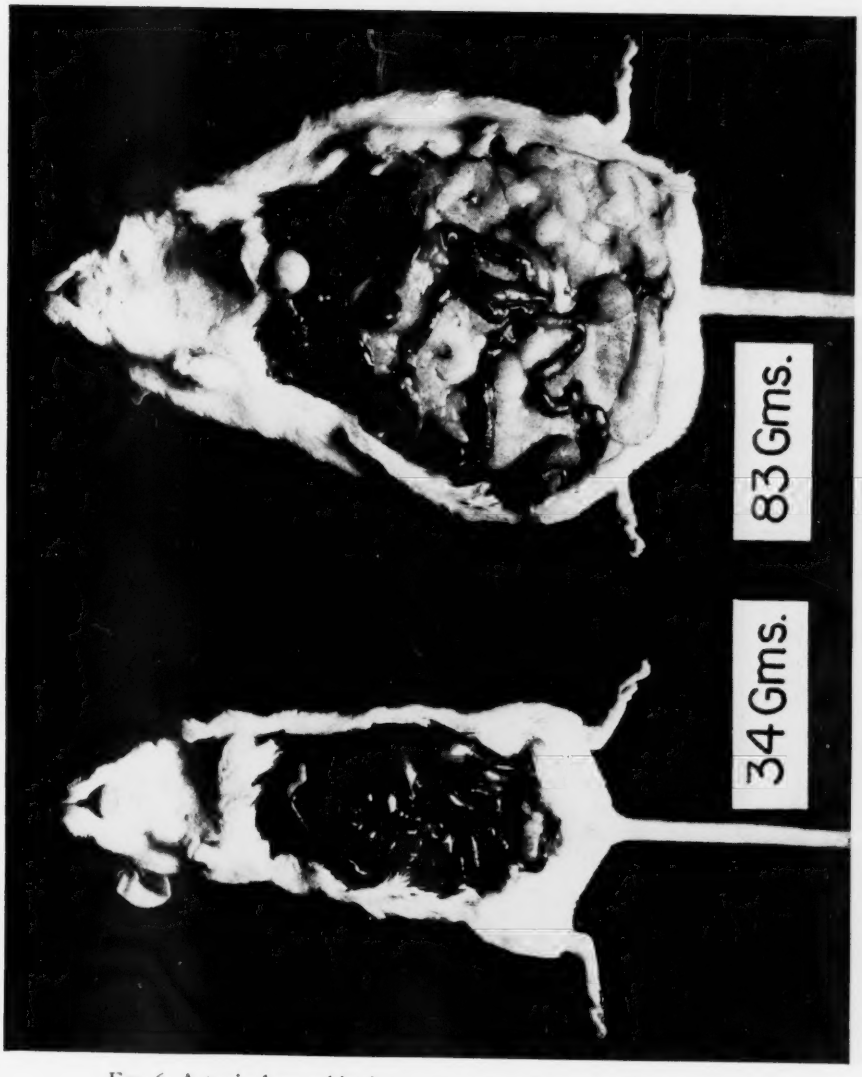


FIG. 6. A typical aurothioglucose mouse and a control at autopsy.

extremely obese mice appeared to be very much larger than in the controls. In many instances the livers of the obese mice were yellow.

Weights of certain representative organs are summarized in Table 4. It will be seen that the proportionate increase in liver, heart, and kidney weight is of the same order of magnitude. It is of some interest that the increase in absolute oxygen consumption for this group of obese mice was about 79 per cent over the control value.

TABLE 4. ORGAN WEIGHTS AT AUTOPSY IN A REPRESENTATIVE GROUP OF TEN OBESE MICE AND TEN CONTROLS. FIGURES IN PARENTHESES DESIGNATE RANGE

	Weight in grams		
	Controls	*Obese*	*Per cent of control*
Body	29.1 (24.5-32.5)	72.1 (61.5-82.5)	248
Liver	1.75 ± S.E. 0.09 (1.4-2.3)	3.0 ± S.E. 0.22 (1.9-4.0)	172
Heart	0.117 (0.095-0.146)	0.191 (0.162-0.210)	163
Kidney	0.337 (0.288-0.400)	0.578 (0.496-0.676)	172
Ovaries	0.010 (0.006-0.020)	0.014 (0.008-0.024)	*

* No significant difference.

Histological examination of hematoxylin and eosin-stained sections of the hearts and kidneys of obese mice and controls revealed no obvious differences between the groups.

The only other autopsy finding of interest was the observation that the mean weight of the ovaries of obese mice was 14.7 mg. (range 8-24) while that of the controls was 10.0 mg. (6-20). This is not regarded as a statistically significant difference.

DISCUSSION

Aurothioglucose is an analogue of glucose; its structure is shown in Figure 7. That a single large dose of the compound in the mouse can produce a marked increase in spontaneous food intake with the subsequent development of obesity there can be no doubt. There is no evidence to sup-

port the view that the obesity is due to any cause other than the increased food intake. Thus, it is currently believed that ATG poisoning can best be visualized as a biochemical lesion of certain cells which have a critical function in the normal regulation of intake to energy expenditure. The anatomical location of these cells and their precise function are quite unknown. Brecher[8] and Haymaker[18] report that extensive examination of the brains of obese mice failed to reveal any lesions which might help to explain the obesity. It is possible that re-investigation of this problem by means of recently developed sulfhydryl staining techniques will yield new and useful information.

Brecher[1] and Owen, *et al.*[24] agree that prior starvation reduces mortality from ATG, and the present results are in agreement with those of Owen, *et al.* insofar as they indicate an increased obesity incidence among the survivors in starvation studies. The mechanism of the increased resistance to the toxicity of ATG associated with starvation is not known. Three possibilities may be considered: (i) it may be related to the decrease in total metabolism incident to starvation, (ii) it may be related to the shift from a predominantly carbohydrate to a predominantly fat metabolic mixture, a shift which might be accompanied by some alteration in function of the "satiety chemoreceptors" which would make them more vulnerable to the gold compound, or (iii) higher concentrations of the toxic material may gain access to critical cells in the fed state than in the fasting state.

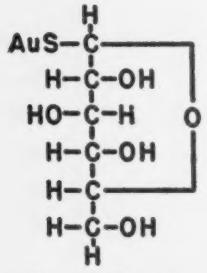


FIG. 7. The structure of aurothioglucose.

In order to examine the possibility that a predominantly fat metabolic mixture might have contributed to the observed result in the starvation experiment, the fat-feeding experiment reported above was done. The markedly diminished survival rate in the fat-diet-adapted group clearly discredits the hypothesis that a fat metabolic mixture in some way renders the tissue less vulnerable to the action of ATG.

These experiments together, however, suggest that the hunger-satiety apparatus may be a far more intricate system than any that has been suggested up to this time. For any satisfactory theory of regulation of food intake to energy expenditure not only must take into account the fact that animals can perform this regulation on diets of widely varying composition but also the possibility that the mechanisms for perceiving satiety may undergo adaptation to different dietary regimens, and that, as suggested

by the work of Lundbaek and Stevenson,[17] their vulnerability to chemical attack may vary in different nutritional states.

The fact that the normal mouse or rat increases the volume of his food intake when his food is diluted with non-caloric bulk materials[24, 14] is indicative of the exquisite sensitivity of the hunger-satiety complex. The failure of the aurothioglucose mouse to increase an already high volume of food as found by Owen, *et al.*[24] should not necessarily be interpreted as a failure of an hypothetical "volume-regulatory" food intake mechanism. It may indicate nothing more than the possibility that the obese mouse has already expanded his volume intake to his maximum capacity, and that there is a mechanical limit beyond which he can increase it no further.

The experiments with BAL reported above failed to dissociate the lethality of ATG from its obesity-producing potency. There is at least strong presumptive evidence that a heavy metal is involved in the obesity-producing lesion. This, in turn, suggests the possibility that the hypothetical satiety chemoreceptors contain some important sulfhydryl-containing protein which is more or less permanently inactivated by the gold compound. ATG obesity, then, is a pharmacological oddity: from the experimental facts it is possible to infer something about its biochemical locus of action before anything whatever is known about its anatomical locus of action.

The experiment in which hyperglycemia was associated with enhanced susceptibility to ATG makes it difficult to postulate a competitive relationship between glucose and its sulfur and gold-containing analogue. It is suggested that some physiological event associated with the induced hyperglycemia, possibly insulin secretion, may have facilitated the entrance of ATG into cells. This hypothesis is being tested currently in studies of the tissue distribution of gold following injection of aurothioglucose under various circumstances.

The difficulties of distinguishing cause from effect in physiological studies of obese animals and their controls are well known to most students of experimental obesity. ATG obesity in mice affords an opportunity to search for common denominators in different experimental situations in which one finds markedly increased food intakes. In the light of reported studies on hypothalamic obesity in rats,[20, 9, 4] some of the sequelae of obesity in the ATG syndrome were regarded primarily as adaptive responses to long continued, excessive food intake.

In these experiments the obese mouse, like the obese rat with an intact pancreas, did not exhibit markedly impaired glucose tolerance.[6] However, all of these studies were made within five months of the time of injection.

It is entirely possible that studies of older obese mice will reveal substantial impairment of glucose tolerance.

Like the hypothalamic obese rat,[5] ATG obese mice show accumulations of fat in the liver and a statistically significant increase in serum cholesterol concentration. Since not only cholesterol but also serum lipoproteins have been shown to be elevated as a result of acute overfeeding in the human,[32] these findings may be regarded as an indication of a common biochemical response to at least three different types of overeating. The mechanism involved is unknown. We suggest the possibility that enhanced lipogenesis from carbohydrate is an adaptive response to overnutrition, and that the total lipotropic capacity of the animal may be overwhelmed by the rate of carbohydrate-to-fat transformation in markedly obese animals. In this connection, it is interesting to note that the livers of obese patients often show fatty infiltration and impairment of function.[34]

The present experiments on the subject of the "hypermetabolism" of obesity require little comment. The splanchnomegaly described in the obese mice was not seen in ATG-injected mice which did not become obese. Since there was a suggestion that rats with hypothalamic obesity[5] also demonstrated some tendency toward splanchnomegaly in spite of the fact that they were not very obese in comparison with the mice used in the current study, the splanchnomegaly may be regarded as one of the adaptations to a persistently high food intake. While this may not be exactly what Grafe[11] had in mind when he postulated his theory of "luxuskonsumption," this does, in fact, represent a form of "luxuskonsumption" and it has the effect of limiting the further development of obesity. It is pertinent, in this connection, to recall that rats with hypothalamic obesity were found to excrete about 35 per cent more creatinine per day than did their litter mate controls,[29] thus indicating that muscle mass may be increased in association with overnutrition and obesity. There was no evidence of skeletal growth in these fat mice, but it must be recalled that they had practically attained their adult size at the time of injection. Attempts are now being made to produce this syndrome in very young rapidly growing mice in an effort to ascertain whether or not skeletal growth can be affected by markedly increased food intake.

There are two rather striking differences between ATG obesity in mice and hypothalamic obesity in the rat that are worthy of comment. First, histological examination of the kidneys of these animals revealed no lesion comparable to that seen in rats with hypothalamic lesions.[5] This is of interest in the light of Stevenson's[27] opinion that the renal lesion in hypothalamic rats is related to the hypothalamic lesion rather than to an alteration in food

habit. Second, ATG obese mice do not show ovarian atrophy, while hypothalamic-operated rats, whether obese or non-obese, frequently did.[8] Again, this suggests the possibility that the ovarian atrophy in the rat with hypothalamic lesions may have been the result of the lesion rather than of overnutrition and obesity.

SUMMARY

1. A single injection of a large dose of aurothioglucose (ATG) may result in a very large increase in spontaneous food intake and marked obesity in the mouse. This is in confirmation of the original observation of Brecher and Waxler.

2. Fasting prior to injection increased both the survival rate and the incidence of obesity among survivors under the conditions of these experiments.

3. Adaptation of mice to a high fat diet prior to injection markedly enhanced the toxicity of ATG when the material was injected into non-fasted animals.

4. When mice were treated simultaneously with ATG and progressively increasing doses of BAL, there was a progressive increase in survival rate and a concomitant decrease in obesity incidence among the survivors.

5. When fasted mice were injected with ATG at the height of an hyperglycemia induced by the intraperitoneal injection of glucose they showed a greatly diminished survival rate as compared with normoglycemic fasted mice.

6. The blood glucose and glucose tolerance of ATG obese mice were not significantly different from those of appropriate controls.

7. ATG obese mice exhibited statistically significant increases in liver fat concentration and in serum cholesterol concentration.

8. The absolute oxygen consumption of obese mice was found to be about 79 per cent greater than that of controls of normal weight. The oxygen consumption of their liver slices per mg. of nitrogen was found to be precisely the same as the corresponding value for control mice. However, it was found that the livers, hearts, and kidneys of the obese mice weighed between 60 and 80 per cent more than did the respective control organs. The "hypermetabolism" of obesity is therefore interpreted as being due to an increase in "active metabolic mass," which may have occurred as an adaptive response to overnutrition and obesity.

9. Certain points of similarity and difference between ATG obesity in the mouse and hypothalamic obesity in the rat are discussed.

ACKNOWLEDGMENT

The authors are indebted to Miss Janet M. DeWitt, who performed the serum cholesterol analyses; to Mr. Gerald Friedman, who gave valuable assistance during the Warburg experiments; and to Dr. David Jones of the Department of Pathology, who helped in the examination of histological material.

REFERENCES

1 Brecher, G.: Personal communication, 1950.

2 Brecher, G.: Personal communication, 1952.

3 Brecher, G. and Waxler, S. H.: Obesity in albino mice due to single injections of goldthioglucose. Proc. Soc. Exp. Biol., N. Y., 1949, *70,* 498.

4 Brobeck, J. R.: Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol. Rev., 1946, *26,* 541.

5 Brobeck, J. R., Tepperman, J., and Long, C. N. H.: Experimental hypothalamic hyperphagia in the albino rat. Yale J. Biol., 1943, *15,* 831.

6 Brobeck, J. R., Tepperman, J., and Long, C. N. H.: The effect of experimental obesity upon carbohydrate metabolism. Yale J. Biol., 1943, *15,* 893.

7 Brody, S.: *Bioenergetics and growth.* New York, Reinhold Publishing Corp., 1945, pp. 329-334, 374.

8 Dickerson, V. C., Tepperman, J., and Long, C. N. H.: The role of the liver in the synthesis of fatty acids from carbohydrate. Yale J. Biol., 1943, *15,* 875.

9 Dublin, L. I., Lotka, A. J., and Spiegelman, M.: *Length of life.* New York, Ronald Press Co., 1949, pp. 193-196.

10 Fisher, R. A.: *Statistical methods for research workers.* New York, Hafner Publishing Co., 10th ed., 1946.

11 Grafe, E.: Probleme der Gewebsatmung. Deut. med. Wschr., 1925, *51,* 640. Cited in Kleiber, M.: Body size and metabolic rate. Physiol. Rev., 1947, *27,* 511.

12 Haldane, J. S.: A new form of apparatus for measuring the respiratory exchange of animals. J. Physiol., Lond., 1892, *13,* 419.

13 Haymaker, W.: Personal communication, May, 1953.

14 Kennedy, G. C.: The hypothalamic control of food intake in rats. Proc. R. Soc. Lond., 1950, *137,* 535.

15 Koch, E. C. and McMeekin, T. L.: A new direct nesslerization micro-Kjeldahl method and a modification of the Nessler-Folin reagent for ammonia. J. Am. Chem. Soc., 1924, *46,* 2066.

16 Lande, K.: Die günstige Beeinflussung scheichender Danerinfekte durch Solganal. Munch med. Wschr., 1927, *74,* 1132. Cited in Comroe, B. I.: *Arthritis and allied conditions.* Philadelphia, Lea and Febiger, 1949, p. 1108.

17 Lundbaek, K. and Stevenson, J. A. F.: Reduced carbohydrate intake after fat-feeding in normal rats and rats with hypothalamic hyperphagia. Am. J. Physiol., 1947, *151,* 530.

18 Mayer, J.: The glucostatic theory of regulation of food intake and the problem of obesity. Bull. N. England M. Center, 1952, *14,* 43.

19 Mayer, J.: Glucostatic mechanism of regulation of food intake. N. England J. M., 1953, *249,* 13.

20 Mayer, J., Russell, R. E., Bates, M. W., and Dickie, M. W.: Metabolic, nutritional, and endocrine studies of the hereditary obesity-diabetes syndrome of mice and mechanism of its development. Metabolism, 1953, *2,* 9.

21 Mayer, J. and Silides, D. N.: A quantitative method of determination of the diabetogenic activity of growth hormone preparations. Endocrinology, 1953, *52,* 54.

22 Nelson, N.: A photometric adaptation of the Somogyi method for determination of glucose. J. Biol. Chem., 1944, *153,* 375.

23 Newburgh, L. and Conn, J.: A new interpretation of hyperglycemia in obese middle aged persons. J. Am. M. Ass., 1939, *112,* 7.

24 Owen, J. A., Jr., Parson, W., and Crispell, K. R.: Dietary dilution studies in goldthioglucose induced obesity in the mouse. Metabolism, 1953, *2,* 362.

25 Payne, R. W.: Studies on the fat-mobilizing factor of the anterior pituitary. Endocrinology, 1949, *45,* 305.

26 Sperry, W. M. in Macy, I. G.: *Nutrition and chemical growth in childhood.* Vol. I., Springfield, Ill. Charles C Thomas, 1942, pp. 331-337.

27 Stevenson, J. A. F.: Effects of hypothalamic lesions on water and energy metabolism in the rat in G. Pincus, Ed. *Recent progress in hormone research.* Vol. IV., New York, Academic Press, 1949, pp. 363-394.

28 Strang, J. M. and Evans, F. A.: The energy exchange in obesity. J. Clin. Invest., 1928-29, *6,* 277.

29 Tepperman, J., Brobeck, J. R., and Long, C. N. H.: Unpublished observations, 1942.

30 Tepperman, J., Brobeck, J. R., and Long, C. N. H.: The effects of hypothalamic hyperphagia and of alterations in feeding habits on the metabolism of the albino rat. Yale J. Biol., 1943, *15,* 855.

31 Wadd, W.: *Cursory remarks on corpulence or obesity considered as a disease.* London, J. Callow, 1816.

32 Walker, W. J.: Relationship of adiposity to serum cholesterol and lipo-protein levels and their modification by dietary means. Ann. Int. M., 1953, *39,* 705.

33 Waxler, S. H. and Brecher, G.: Obesity and food requirements in albino mice following administration of goldthioglucose. Am. J. Physiol., 1950, *162,* 428.

34 Zelman, S.: The liver in obesity. Arch. Int. M., 1952, *90,* 141.

FREDERICK G. KILGOUR | *Yale Medical Library, Yale University School of Medicine*

WILLIAM HARVEY'S USE OF THE QUANTITATIVE METHOD*

In the extensive literature on the discovery of the concept of the circulation of the blood appear many claims that various men other than William Harvey (1578-1657) were the true discoverers. Bayon[1] discusses the case for the following six: Andrea Cesalpino (1524-1603), Realdo Colombo (1510-59), Helvicus Dietericus (1601-55), Galen (131-201), Paolo Sarpi (1552-1623), and Walter Warren (d. 1640); in addition, there have been claimants for François Rabelais (1485-1553), Carlo Ruini (d. 1598), and Michael Servetus (1509-53). Apparently, as a kind of hedge against fate in the event that it turned out that someone else had discovered the circulation earlier, some of Harvey's proponents have also attempted to establish him as the "Father of Experimental Physiology," and others have suggested that his introduction of quantitative methods into biology was more important than his disclosure concerning the circulation.

There is no doubt that Harvey both developed and adequately demonstrated his great concept of the function of the heart and the motion of the blood. Historically, it is clear that the importance of Harvey's work was his hypothesis of the circulation; his observations on the motion of the heart and blood, while important, have had less subsequent effect on physiology than his concept. Using symbolic logic, J. H. Woodger has arrived at the same conclusion.[2] Harvey's concept has continued to be a fruitful one for more than three centuries, and it still suggests investigations to be made. There can also be no doubt that Harvey played an important rôle in reintroducing experiment into seventeenth century biology; but it is most

---

* Presented before the Beaumont Club on 14 March 1952. This paper contains some of the details on which I based my article on William Harvey in the *Scientific American*, 1952, *186*, 57. I am most grateful to Dr. John F. Fulton not only for suggestions which have improved this paper, but more particularly for having aroused my interest anew in historical matters, after seven years of inactivity in such fields, by inviting me late in 1948 to discuss Harvey in his seminar on the history of the circulation.

[1] Bayon, H. P.: William Harvey, physician and biologist: his precursors, opponents and successors—Part V. Ann. Sci., 1939, *4*, 352-371.

[2] Woodger, J. H.: *Biology and language*. Cambridge, University Press, 1952. pp. 45-49, 75-92. I am grateful to Mr. Rulon S. Wells, 3rd of the Yale University Department of Philosophy for having brought Professor Woodger's interesting analysis to my attention.

doubtful that his use of the quantitative method was of as great importance as his concept of the circulation. Although there were a dozen or more noteworthy biological investigations involving quantitation in the seventeenth and eighteenth centuries subsequent to Harvey, these uses of quantitative method were not entirely the result of Harvey's work. Of equal and probably greater importance was the influence of Galileo (1564-1642) both directly and indirectly through the writings of such men as Santorio Santorio (1561-1636) and Giovanni Alphonso Borelli (1609-79). Moreover, biology in general did not become quantitative until the end of the nineteenth century. For instance, Charles Darwin's *Origin of Species* (1859) contains almost no quantitation, and it was typical that one of the foremost investigators of biological inheritance, C. W. Nägeli, should have questioned Gregor Mendel as to whether or not his statistical work was "empirical rather than rational," the implication being that quantitative work was of little value. Nevertheless, Harvey's excellent experimental procedures certainly inspired some seventeenth century biologists to develop his techniques.

Whence did Harvey obtain the idea of the experimental method? He had two principal sources: one, the eminent work of Galen, the other, the new tradition of experimental science which germinated in the thirteenth century and flowered in the seventeenth in Western Christendom. Galen was perhaps the greatest experimentalist in biology, certainly the greatest in Greek science, which embodied little experimentation outside of biology. Harvey knew of Galen's experiments, referred to them, and repeated some of them.[3] The rational procedures of mathematics and logic, together with the empirical and quantitative methods of technology, strongly influenced the evolution of the new theory of experimental science which slowly came into being in northern Europe during the thirteenth and fourteenth centuries.[4] Experimental science reached its maturity in the next two centuries in Italy, particularly at Padua[5] where Harvey studied medicine from 1598 to 1602. Harvey was not the only experimentalist of the early seventeenth century; there were many others, of whom Galileo (1564-1642) was the greatest. Experimentation was in the air.

---

[3] Harvey, William: *Exercitatio anatomica de motu cordis et sanguinis in animalibus. With an English translation and annotations by Chauncey D. Leake.* Springfield, Ill., C. C Thomas, 1928. *Passim.* Unless otherwise noted, references to Harvey's celebrated book are to Chauncey Leake's splendid translation.

[4] Crombie, A. C.: *Robert Grosseteste and the origins of experimental science, 1100-1700.* Oxford, Clarendon Press, 1953.

[5] Randall, J. H.: The development of the scientific method in the school of Padua. Journal of the History of Ideas, 1940, *1*, 177-206.

Most of the experiments in Harvey's *De Motu Cordis* are quite simple but well-thought-out demonstrations involving ligating or severing blood vessels, and the opening of the cardiac auricles and ventricles. One interesting example of Harvey's use of Galen's techniques is his repetition of one of Galen's experiments and his obtaining a different finding therefrom. Galen had maintained that the walls of arteries transmitted the pulse from the heart as the result of his having inserted a reed into an artery and then drawing tight a ligature around that section of the artery containing the reed.[6] Galen reported that before he drew up the noose, the artery beyond the reed continued to pulse, but after he tightened the ligature, the pulse stopped. From this observation, Galen concluded that the pulse was due to a movement passing along the arterial wall and not to the blood within the artery. Apparently, the blood clotted in the reed during the experiment or Galen crushed the reed when he tightened the noose, thereby diminishing the blood flow in the artery beyond the reed. This experiment is more complex than most that Harvey did, and he had trouble with it. The first written record of Harvey's discovery is in a 98-sheet notebook of lecture notes which he prepared for his Lumleian Lectures in April, 1616; in these notes he wrote ". . . Galeni experimentum de fistula Impossible."[7] Twelve years later in the Introduction to his *De Motu Cordis* he stated that he had not done Galen's experiment and that he did not think it possible to do. However, by 1649, when Harvey published his *Exercitatio Anatomica de Circulatione Sanguinis* addressed to Jean Riolan, he had been able to repeat the experiment at least to the extent of showing that there was a diminished pulse beyond the tube when the ligature was tight.[8]

Among the many scientists whose work contributed to establishing experimental biology, Harvey played an important rôle and appears to have directly influenced some of the biologists who followed him. The clearest indication of the consequence of Harvey's experimentation is in the work of those who further investigated the circulation. Many of these men did new experiments which were similar to Harvey's. For instance, Jan de Wale (1604-49) of Leyden reported[9] in a letter to Thomas Bartholin in October 1640 and first published in 1641 on the effect of ligating the pulmonary vein. Wale found that the vein swelled on the side of the ligature away

[6] Galen: An in arteriis natura sanguis contineatur. In *Medicorum Graecorum opera quae exstant*. Edited by C. G. Kühn, Leipsig, 1882. Vol. IV, pp. 733-734.
[7] Harvey, William: *Prelectiones anatomiae universalis*. London, J. & A. Churchill, 1886. s. 79 recto.
[8] Harvey, William: *The circulation of the blood*. New York, Dutton, 1923. pp. 134-136. (This widely available Everyman's edition contains translations originally done for the Sydenham Society in 1848 by Robert Willis.)
[9] Wale, Jan de: *Epistolae duae*. Lugd. Bat., 1647. pp. 39-40.

from the heart and that it emptied between the ligature and the heart, showing that the blood in the pulmonary veins flows from the lungs to the heart. When Harvey published his *De Motu Cordis* in 1628 he did not include any new experimental work on the pulmonary transit which Michael Servetus had so clearly described in his *Christianismi Restitutio* (1553). Servetus' work was unknown during Harvey's lifetime because the Calvinists destroyed most of the copies of it when they burned Servetus at the stake in 1553, but Realdo Colombo had also reported his apparently independent discovery of the transit in his *De Re Anatomica* (1559), three years after one of his students, Juan Valverde, had published his professor's investigations in his *Historia de la Composicion del Cuerpo Humano* (1556). Harvey's arguments for the pulmonary transit in Chapters VI and VII of his *De Motu Cordis* are based largely on Colombo's work to which he added some comparative anatomical evidence of his own. However, in his second letter to Jean Riolan he described new work on the pulmonary transit, and one of the new experiments is similar to Wale's,[10] whose work he knew about.[11]

The French physician, Jean Pecquet (1622-74), who discovered the thoracic duct, made, among other experiments, some on the pulmonary transit.[12] The relatively little known Henry Power (1623-68) of Halifax and New Hall performed various physiological experiments, including one which showed that blood did not pass through the interventricular septum in accordance with the Galenic scheme. Power cut off the left ventricle of a living "dogge" and observed that the contractions of the right ventricle did not ". . . squeese any blood through the septum."[13] Richard Lower (1631-91) did many experiments described in his *Tractatus de Corde* (1669) which are in the tradition of Harveian experimental biology. In addition to Wale, Pecquet, Power, and Lower, there were numerous others whose work Harvey influenced, but the experiments of these four not only are good examples of the influence exerted by Harvey's work but also show that his methods were being used by Dutch and French investigators as well as by physicians in England outside of London. Although it is not correct to imply that Harvey invented or, as the case may be, begot experimental physiology by calling him the "Father" thereof, he nevertheless

---

[10] Harvey: *Op. cit.* (note 7), pp. 164-165.

[11] *Ibid.*, p. 114.

[12] Pecquet, Jean: *Experimenta nova anatomica . . . Eivsdem dissertatio anatomica de circulatione sangvinis, et chyli motu.* Paris, 1651. pp. 34-35.

[13] Rolleston, Humphrey: The reception of Harvey's doctrine of the circulation of the blood in England as exhibited in the writings of two contemporaries. In *Essays on the history of medicine, presented to Karl Sudhoff on the occasion of his seventieth birthday.* London, Oxford University Press, 1924. p. 252.

contributed much to the rise of the experimental attitude in the seventeenth century.

Harvey wrote out his most complete quantitative arguments in Chapter IX of his *De Motu Cordis;* the preceding portion of the book contains introductory sections, four chapters analyzing the motion and function of the heart, two on the pulmonary transit, and one, Chapter VIII, on his concept of the general circulation; he begins Chapter IX by setting forth the following three propositions by which he intended to prove and thereby demonstrate the circulation:

> First, blood is constantly being transmitted from the vena cava to the arteries by the heart beat in such amounts that it cannot be furnished by the food consumed, and in such a way that the total quantity must pass through the heart in a short time.
>
> Second, blood is forced by the pulse in the arteries continually and steadily to every part of the body in a much greater amount than is needed for nutrition or than the whole mass of food could supply.
>
> And likewise third, the veins continually return this blood from every part of the body to the heart.[14]

In other words, Harvey proposed to show (i) that the heart pumped the total amount of blood in a relatively brief time, and that the amount of blood going through the pulmonary transit was so great that the ingested food could not possibly supply it as called for by the then current Galenic concept of the origin and motion of the blood; (ii) that the heart sends blood out through the systemic arteries in much larger quantities than either the body uses for its nutrition or, once again, food can supply; and (iii) that the blood in veins flows only toward the heart and not also away from it as the Galenic theory would have it.

Having stated that the proof of these propositions will make it obvious that blood circulates, Harvey next presented his quantitative arguments showing that the quantity of blood passing through the pulmonary transit is so great that it cannot be produced from the ingested food. The following translation is essentially that of Chauncey Leake with a few changes to make it more literal; it is the only section of Harvey's book that contains specific measurements.

> Let us suppose, by reflection or by experiment, that the left ventricle of the heart when filled in diastole, contains either two ounces or three ounces or an ounce and a half.[15] In a cadaver I have found it holding more than two ounces.

---

[14] Harvey: *Op. cit.* (note 2), p. 73.

[15] All weights are Apothecaries' weight.

3 scruples	=	1 dram
8 drams	=	1 ounce
12 ounces	=	1 pound
1 scruple	=	1.296 g.

Likewise let us suppose how much less the ventricle contains when the heart contracts or how much blood it forces into the great artery with each contraction, for, during systole, everyone will admit something is always forced out as shown in Chapter III, and apparent from the structure of the valves. As a reasonable conjecture suppose a fourth, fifth, sixth, or at least an eighth part is passed into the arteries.

Then we may suppose in man that a single beat would force out[16] either a half ounce, three drams, or even one dram of blood, which because of the valvular block, could not flow back into the heart.

The heart makes more than a thousand beats in a half hour, in some two, three, or even four thousand. Multiplying by the drams, there will be in half an hour either 3,000 drams, 2,000 drams, 500 ounces, or some other such proportionate amount of blood forced into the arteries by the heart, but always a greater quantity than is present in the whole. Likewise in a sheep or a dog, suppose one scruple goes out with each stroke of the heart, then in half an hour 1,000 scruples or about three and a half pounds of blood would pass through the heart.[16] But as I have determined in the sheep, the whole body does not contain more than four pounds of blood.

On this assumption of the passage of blood, made as a basis for argument, and from the estimation of the pulse rate, it is apparent that the entire quantity of blood passes from the veins to the arteries through the heart, and likewise through the lungs.

But suppose this would not occur in half an hour, but rather in an hour, or even in a day, it is still clear that more blood continually flows through the heart than can be supplied by the digested food or be held in the veins at any one time.

It cannot be said that the heart in contracting sometimes propels and sometimes doesn't, or that it propels a mere nothing or something imaginary. This point has been settled previously, and besides, it is contrary to common sense. If the ventricles must be filled with blood in cardiac dilation, something must always be pushed out in contraction, since the passages are not small nor the contractions few. This quantity expelled is some proportion of the contents of the ventricle, a third, a sixth, or an eighth, and an equivalent amount of blood must fill it up in diastole, so that there is a relation between the ventricular capacity in contraction and in dilation. Since the ventricles in dilating do not become filled with nothing, or with something imaginary, so in contracting they never expel nothing or something imaginary, but always blood in an amount proportionate to the contraction. So it may be concluded that if the heart in a single beat in man, sheep, or ox sends forth one dram, and there are 1,000 beats in half an hour, the total amount transmitted in that time would be 10 pounds 5 ounces; if two drams at a single stroke, then 20 pounds 10 ounces; if half an ounce, then 41 pounds 8 ounces; if one ounce, then a total of 83 pounds 4 ounces, all of which would be transferred from the veins to the arteries in half an hour.

In the first part of this section, Harvey used the contents of the *left* ventricle to make his basic assumption as to the amount of blood which the right ventricle ejects for passage through the pulmonary transit. He does not explain why he uses the capacity of the left ventricle instead of the right. It may be, however, that since he had already discussed the pul-

[16] Willis gratuitously inserted "into the aorta" at these two points. See Harvey, *Op. cit.* (note 7), p. 59.

monary transit in Chapters VI and VII, he used the weight of the contents of the left ventricle as being the quantity of blood which had passed through the transit or, as he would have put it, from the veins to the arteries. His use of the left ventricle has caused confusion amongst some of Harvey's interpreters who have presented this section as demonstrating the systemic transit, and it is probably the reason for Willis having twice inserted the phrase "into the aorta" in his translation which was the standard English translation until Dr. Leake's in 1928.

To make his calculation of the amount of blood flowing through the pulmonary transit, Harvey had to measure the pulse rate and the amount of blood which the heart ejects with each beat; the pulse is easy to measure, but even the most modern procedures for measuring cardiac output give results which vary as much as 25 per cent. According to Aubrey, Harvey's brief biographer, Harvey while dying gave one of his nephews the minute watch which he had used in his experiments.[17] Although he mentions pulse rates of 33, 67, 100, and 133 per minute, he used a rate of 33 in the only calculation in which he specifically mentioned man. It is obvious from this that he did not use his minute watch to make this measurement, and it is apparent from his lumping together "man, sheep or ox," all with a pulse of 33, which is reasonably correct for an ox but only half the correct figure for man or sheep, either that he did not take the trouble to measure their pulse rates, or that he was not particularly concerned about using the correct rate. Of the two alternatives, the latter is the more likely.

When it came to measuring cardiac output, Harvey was obliged to guess at a measurement which has not yet been accurately determined, but he might have been more accurate than he was. The only two specific measurements of the weight of blood that he records are the "more than two ounces" he found in the left ventricle of a cadaver and the "not . . . more than four pounds," which he obtained by exsanguinating a sheep. This figure of four pounds is, of course, low, because by using the rudimentary techniques available to him, he could not have drawn off all the blood. His estimates of the stroke volume as being either ¼, ⅕, ⅙, or ⅛ of the ventricular content are sheer guesses. Although it would have been impossible for Harvey to arrive at an approximately accurate value of the cardiac output in man, he could have done much better in the case of sheep. If he had severed the aorta of a sheep and had weighed the amount of blood ejected during 10 or 20 heart beats, he could have obtained a fairly accurate figure for the stroke volume. Presumably, he never made this observation

[17] Aubrey, John: *Aubrey's brief lives.* London, Secker and Warburg, 1949. p. 132.

or one similar to it, such as Richard Lower's discussed below, because he did not feel that he had to be particularly accurate.

This conclusion is reinforced by an inspection of Table 1, which gives all the possible values for stroke volume in man based on Harvey's assumed values of ventricular capacity and proportion of contents ejected by each contraction. It should be noted that one dram is not one of the possible

TABLE 1. POSSIBLE VALUES FOR STROKE VOLUME IN MAN BASED ON HARVEY'S ASSUMPTIONS

Estimated contents of left heart		*Proportion of contents ejected by each contraction*		*Weight of blood ejected by each contraction**
1.5 oz.	×	1/8	=	1.5 dr.
1.5 oz.	×	1/6	=	2 dr.
1.5 oz.	×	1/5	=	2.4 dr.
1.5 oz.	×	1/4	=	3 dr.
1.5 oz.	×	1/3	=	4 dr.
2 oz.	×	1/8	=	2 dr.
2 oz.	×	1/6	=	2.67 dr.
2 oz.	×	1/5	=	3.2 dr.
2 oz.	×	1/4	=	4 dr.
2 oz.	×	1/3	=	5.33 dr.
3 oz.	×	1/8	=	3 dr.
3 oz.	×	1/6	=	4 dr.
3 oz.	×	1/5	=	4.8 dr.
3 oz.	×	1/4	=	6 dr.
3 oz.	×	1/3	=	1 oz.

* All values are apothecaries' weight.

values, yet Harvey used it in his first and third calculation. Either he did not do the arithmetic in Table 1, or he merely rounded off one and a half drams to one dram. Moreover, he included sheep in his second and third calculations and employed variously a cardiac output of one scruple (1.3g), one dram (3.9g), two drams (7.8g), half an ounce (15.6g), and one ounce (31.1g). Certainly Harvey was not concerned with accurate measurement.

Harvey concluded Chapter IX by writing that he would next discuss anastomosis between veins and arteries, "but he doesn't" as Chauncey Leake pointed out in a footnote. However, Harvey did write about anastomsis in the first paragraph of Chapter XI so it seems clear that he inserted

Chapter X after having written what is now XI to follow IX. Someone had apparently raised an objection to his idea of the circulation by claiming that the blood flowed out of the body in the form of other fluids such as milk from mammals. In the new chapter Harvey answered this criticism by saying that by computation the heart pumps in an hour or less more than all the milk produced in a day, but he did not do any computation.

Chapters XI and XII are devoted to the proof of the second proposition that the ". . . blood is forced by the pulse in the arteries continually and steadily to every part of the body in a much greater amount than is needed for nutrition or than the whole mass of the food could supply." Actually, Chapter XI consists almost entirely of descriptions of his well-known experiments using ligatures on limbs to show that the blood passes from the arteries to the veins. In a sense, most of the chapter deals with anastomosis, but there is no quantitative work in it. One paragraph of Chapter XII, however, does contain quantitative arguments for the systemic transit. Harvey wrote

> . . . we may very readily compute the amount of blood and come to some conclusion on its circular motion. If, for instance, in phlebotomy, one were to let the blood flow with its usual force and rate for a half hour, there is no doubt but that the greater part of it would be drained off, practically emptying not only arteries but also the great veins, and that fainting and syncope would follow. It is reasonable to assume that as great an amount of blood as is lost in this half hour's time, passed from the great veins through the heart to the aorta. Further, if you figure how many ounces of blood flow through a single arm, or pass under a medium bandage in twenty or thirty heart-beats, you will have a basis for estimating how much flows through the other arm in the same time, or through both sides of the neck, or through both legs, and through all the other arteries and veins of the body. Since all these are continually supplied with fresh blood, which must flow through the lungs and ventricles of the heart, from the veins, it must be accomplished in a circuit, since the amount involved is much more than can be furnished from the food consumed, or than is needed for the nourishment of the parts.

Harvey did not make the suggested computation, and the statement hardly seems adequate as a quantitative demonstration of the systemic transit. Although he made measurements, albeit not precise ones, to prove the demonstration of the pulmonary transit, Harvey made no measurements to prove quantitatively the systemic transit.

His third proposition Harvey proved in Chapter XIII by using his excellent demonstration of the valves in the veins and of the direction of the blood flow in veins. At the end of the chapter he produced another quantitative argument much like that in Chapter XII. After directing the reader to expel the blood from the section of a vein between two valves, to

allow the section to refill and to repeat the procedure ". . . a thousand times as quickly as possible," he then says, "By careful reckoning, of course, the quantity of blood forced up beyond the valve by a single compression may be estimated, and this multiplied by a thousand gives so much blood transmitted in this way through a single portion of the veins in a relatively short time, that without doubt you will be very easily convinced by the quickness of its passage of the circulation of the blood." But once again he does not make the estimate or the computation, and uses the quantitative argument only by inference, which is hardly what is understood by the phrase "quantitative biology."

These instances of quantitation are the only known examples of Harvey's methods that involve weights. There may have been others in his manuscripts that Civil War rioters destroyed when they ransacked his London house in 1642, or in the eleven now unknown "treatises" to which Charles Goodall[18] referred in 1684 as though they were in existence at that time. Despite these possibilities, there is no evidence of quantitative work by Harvey, other than the above examples, that had any influence on subsequent biologists.

The reason why Harvey was not more precise in his measurements was that he did not have to be to demonstrate his great discovery. Using his lowest estimates for stroke volume and pulse rate which for two or more beats give a cardiac output only 1/36 that of the lowest figure accepted today, Harvey could have shown that in ten hours the heart would eject an amount of blood weighing more than the average man. If Harvey had been trained to use instruments of precision to control quantitative methods, he probably would have used an accurate pulse rate, but otherwise it is likely that his calculations would have been the same. Today's more accurate measurements of cardiac output do not change Harvey's conclusion that the blood circulates.

The origin of Harvey's quantitative attitude is unknown; it is quite likely that he adopted the well-known techniques of measuring with weights and balances as a result of his own reasoning. Men had been using weights as a form of measure for at least 4,500 years, and one branch of medicine, pharmacy, had been compounding remedies by using weights for centuries. Apparently, quantitative evidence was not important in leading Harvey to develop the idea of the circulation because there is no quantitation in his Lumleian Lecture notes of 1616. Scientists and physicians were just beginning to adopt quantitative methods in investigation, although, as Cyril

[18] Goodall, Charles: *The Royal College of Physicians of London.* London, M. Flesher, 1684. Signature Ss1*b*-Ss2*a*.

Stanley Smith[19] has pointed out in the case of assayers, some types of sixteenth century technologists were thoroughly quantitative in their outlook. Galileo was teaching at the University of Padua during the years that Harvey was a student, and it seems almost impossible that Harvey could have completely avoided acquaintance with Galileo's principles of measurement. The Universita Artista, that section in which Galileo taught and which contained the medical school, probably had not more than one or two hundred students enrolled during Harvey's student years. There were fewer than one hundred enrolled in the 1630's,[20] the earliest period for which enrollment records exist. In such a small student body, Harvey surely must have known something of Galileo's work, but there is no direct evidence that he did.

Under Galileo's influence, Santorio Santorio (1561-1636) was the first to make extensive use of precision instruments to control observations in biology and medicine. He described his pulsologium for measuring the pulse rate in 1620 and a thermometer for measuring body temperature in 1612.[21] Santorio published his celebrated measurements of the insensible perspiration by the use of balances in 1614.[22] Reprinted nearly forty times in the original Latin and in translations during the next one hundred and fifty years, Santorio's *De Statica Medicina* played a much larger rôle than Harvey's *De Motu Cordis* in stimulating the use of the balance in biology. It is interesting to note that a repetition of Santorio's work was the only quantitative physiology using the balance that was done in the British colonies of America and published in the *Philosophical Transactions* of the Royal Society.[23] That the Scientific Revolution should have occurred when it did has not been explained, and unfortunately the history of the interrelationships among the technologies, the arts, and the sciences during the latter part of the sixteenth century has not yet been written so that general influences which may have inspired Harvey to use quantitative arguments cannot yet be evaluated. Nevertheless, at Padua quantitation must certainly have been under discussion while Harvey was there, and like experimentation, it was at least in the Paduan air.

---

[19] Ercker, Lazarus: *Treatise on ores and assaying.* Translated . . . by A. G. Sisco and C. S. Smith. Chicago, University of Chicago Press, 1951. pp. xv-xvi.

[20] Saibante, M., Vivarini, C., and Voghera, G.: Gli studenti dell'Università di Padova della fine del 500 ai nostri giorni (studio statistico). Metron, 1924-25, *4*, 177.

[21] Castiglioni, Arturo: Life and work of Sanctorius. Medical Life, 1938, *38*, 753-756.

[22] Santorio, Santorio: *De statica medicina.* Lipsiae, G. Ritssch, 1614. *Passim.*

[23] Lining, John: Extracts of two letters . . . giving an account of statical experiments . . . Philos. Tr. R. Soc. Lond., 1742-43, *42*, 491; also A letter serving to accompany some additions to his statical experiments. *Ibid.*, 1744-45, *43*, 318.

In addition to the work of Harvey and Santorio, the best known and most important quantitative research in biology in the seventeenth century was that of Jean Baptiste van Helmont (1577-1644),[24] Richard Lower (1631-91),[25] and Giovanni Alphonso Borelli (1608-79).[26] There is every reason to believe that Harvey had no influence on van Helmont's celebrated experiment of weighing a growing tree and the earth in which it was planted,[27] but Harvey's work most certainly had some effect on Lower's experiments. Lower's most important contribution to quantitative investigation of the circulation was the use of the total weight of the blood in the left ventricle as being the amount ejected during systole, instead of some fraction thereof as Harvey had assumed. However, Lower did not arrive at this conclusion by using quantitative methods. Rather he observed that when he cut off the tip of a living heart and inserted his little finger, the ventricle closed so completely during systole that he could not squeeze the walls of a heart more tightly together on the finger.[28] From this observation, he concluded that the entire contents of the left ventricle were ejected with each contraction, but he made no measurements of the stroke volume. He did, however, weigh the contents of left hearts removed from cadavers and found they often held much more than two ounces of blood. Nevertheless, in his calculations he used Harvey's measure of two ounces. Lower's method of quantitation was no advance over Harvey's.

Borelli used three ounces as the stroke volume in his remarkable analyses of the dynamics of the circulation,[29] but it is not clear as to just how he arrived at this figure. Much of Borelli's book consists of excellent quantitative experiments in which he consistantly used the balance and occasionally the thermometer. He not only used precision instruments for control, but also did many geometrical analyses, even of the heart, which are totally lacking in Harvey. Borelli's work, of course, is completely within the Galilean tradition, and it is most unlikely that Harvey's less sophisticated methods had any important effect on him.

It seems clear from the above analysis that Harvey's elementary quantitation, although adequate for his purposes and influential on a few biological investigators of the seventeenth century, had less effect than is often represented. His concept of the circulation stands by itself as his greatest achievement.

---

[24] Helmont, Jean Baptiste van: *Ortus medicinae.* Amsterdam, Elzevir, 1648.
[25] Lower, Richard: *Tractatus de corde.* London, J. Redmayne, 1669.
[26] Borelli, Giovanni Alfonso: *De motu animalium.* Rome, A. Bernabò, 1680-81. 2v.
[27] Helmont: *Op. cit.* (note 24), p. 109.
[28] Lower: *Op. cit.* (note 25), p. 154.
[29] Borelli: *Op. cit.* (note 26), v. 2, p. 99.

HENRY BUNTING 1911 - 1954

It is presumptuous for any one person to claim to know another. It is especially so for me to claim to have known, in any total sense of the word, Henry Bunting. For, as I look around this assembly of friends or recall the variety of those who have spoken to me about him during the last few days, I realize how deep, yet how widely-ranging, was his power of friendship.

I grew up with him, amid the peaceful, almost cloistered atmosphere of a college town where our families were friends and colleagues; we went on together to school and university where we both eventually returned to share alike the teacher's life and the exciting delights of rural living in New England. Others here have known him as a fellow worker on those scientific frontiers whose boundaries he was doing so much to extend. To the younger ones he was the professor and practitioner, ever-present and ever-willing to share his learning. Still others have known him in his home or in theirs, or in the hurly-burly of town meeting, or sharing the delights that only music can bring.

What was it that we all found in him that has brought us here today? At first sight one recalls his many-sidedness, his versatility. Only yesterday I opened a student's folder, as we sat discussing his future, to have fall out a letter of Henry's, describing at length a series of interviews during which Henry and the boy had patiently worked together to try to determine the latter's fitness for medicine. Beside the teacher, but never submerging him, was the scientist-scholar—which not only gave Henry his profession, but also permeated his every attitude toward life. Yet of all people he was no disembodied scientist, barely tolerating the outside world, and always impatient to return to the splendid isolation of his laboratory. A dozen other Henrys come to mind; the puckish host or guest, delighted to take off in some extravagant fantasy or paradox; the voice of common sense and public spirit, rising above the petty cries of private interest or short-termed advantage; the silent support of a half-dozen causes and as many more individuals who never quite perceived, perhaps, what strength they derived from his backing; the lover and preserver of nature in every form and mood, her winter silences, her wooded retreats, her creatures large and small. Yet this curiosity, this interest, this awareness was constantly maintained without any feverish timetable or little book to regulate and apportion it. Whenever and wherever I met Henry, I somehow felt immediately that he actually had nothing else to do but to sit and talk to me, even though a moment's reflection would have reminded me that he had probably taught a couple of classes already that day, was dining in town to keep watch on something in the laboratory, had probably begun the day helping Charles

HENRY BUNTING 1911-1954

to build a chicken coop, and would greet the morrow, in the cold dawn, out bird-watching with Polly.

Whatever our interests and our separate problems (and his sympathy was only rivalled by his understanding), we all found in and with Henry something in common. He was a modest man—which always kindled our feeling that he was with us in spirit as well as in body. He was a moderate man, verging when necesary on the imperturbable, always a virtue in a physician. He was a fair man, possessed of that wisdom which lingers long past knowledge, able to understand all points of view yet quietly persuaded where he himself was to stand firm. But informing all those more outward traits of character was an essential honesty which pervaded everything he did. Animals always recognized it, for they had no truer friend. Children saw it when they were hurt, he was no more concerned than the accident really warranted; and I think all of us felt it, like a breath of fresh air in a heated room or sanity in an affected world; we knew exactly where he stood, we felt he knew and sympathized with our position.

I suspect this honesty was possible, ultimately, because of the utter selflessness on which it rested. If private interest lurks around the corner, it is so easy to rationalize a retreat. Not with Henry. He never counted the cost or estimated what his score might prove to be. Like the medieval ascetic, Henry almost shunned material possessions and for the same reasons. They tended to come between him and others, to diffuse that warmth which he gave off so lavishly. Polly has told me of a letter, written years ago when they were newlyweds and his family was urging him to buy a house. He resisted, saying "the only kind of possession that has ever seemed real to me is appreciation; in this sense my holdings are very considerable and all my titles are clear."

THOMAS C. MENDENHALL

Editorial Note. The death on April 15th of Dr. Henry Bunting, Associate Professor of Pathology, brought sorrow to a large circle of family, neighbors, students, fellow faculty members, and other friends in many places. His life-long friend, Thomas C. Mendenhall, Master of Berkeley College and Associate Professor of History, spoke for all of these in this appreciation read at a memorial service in Dwight Chapel on April 22, 1954.

EVENTS

YALE MEDICAL SOCIETY

March 8, 1954

ULTRASONIC MEASUREMENT OF BLOOD VISCOSITY. By Raymond Yesner and Alfred Hurwitz, Veterans Administration Hospital, West Haven, Connecticut.

A new technique for continuous measurement of viscosity is presented with an analysis of viscosity curves from 67 normal healthy adults, and from 10 normal adults, before and after 50 mg. of intravenously injected heparin. Within a few seconds after administration, the effects of heparin are noted in prolonging clotting time and flattening of the blood viscosity profiles. The return of clotting time and viscosity profiles to normal occurs first in the heavier group, indicating that the dosage of intravenous heparin should be varied with the individual's weight. In all cases the clotting time returned to normal prior to the blood viscosity profile. A method of decalcifying blood has also been devised, the results of which closely parallel those obtained with fresh blood. Such curves are reproduced in eight different normal and pathological situations.

TRUMBULL LECTURE

February 18, 1954

THE INDUCED BIOSYNTHESIS OF AN ENZYME. By Jacques Monod, Service de Physiologie Microbienne, Institut Pasteur, Paris.

The mode of synthesis of the *Escherichia coli* enzyme β-galactosidase (lactase) and its relationship to protein formation in general have been studied. β-galactosidase, a so-called adaptive enzyme, was induced by a variety of galactosides. Not all inducers were substrates, and substrates were not necessarily inducers. β-galactosidase, which hydrolyzes β-galactosides to galactose and the aglycone, was found to have a molecular weight of over 500,000 and in fully induced cells comprised approximately 5 per cent of the total cell protein.

Two processes are involved in the synthesis of an adaptive enzyme: the induction process which may correspond to the formation of an intracellular "organizer" and, secondly, the actual synthesis of the enzyme from amino acids. It was found that for every one molecule of inducer used, a considerable number of molecules of enzyme were formed and it was concluded that the inducer must act in a catalytic manner. Adaptation could be shown to involve the synthesis of a new protein. This was demonstrated by the appearance of a new immunogenic cell component, its increase quantitatively paralleling adaptation. Unadapted cells contained a closely related, enzymatically inactive protein. Although the concentration of this latter protein decreased during adaptation, it is probably not a precursor of β-galactosidase.

Most of the experiments were carried out under "conditions of gratuity" where the presence or absence of the enzyme β-galactosidase had no obvious effect on cell growth. When a primary inducer, e.g. methyl-thio-β-D-

galactoside, was added to a logarithmically growing population, β-galactosidase formation started almost instantaneously at a maximum and constant rate. Furthermore in the presence of these same inducers the amount of β-galactosidase synthesized constituted a constant proportion of the total protein formed at all times.

A number of experiments in which isotopically labeled sulfur was employed demonstrated the stability of β-galactosidase; its synthesis is an essentially irreversible reaction. No exchange between β-galactosidase and other cell proteins occurred under a wide variety of conditions and no evidence for a "dynamic state" of proteins in E. coli was found. It was suggested that the turnover of proteins, reported to take place in mammalian tissue, might reflect cell breakdown and resynthesis, secretion etc., rather than protein instability and exchange reactions on an intracellular, molecular level.

P. B.

TERRY LECTURES

March 9, 10, 11, 1954

BECOMING. THREE LECTURES ON GROWTH IN PERSONALITY. By Gordon W. Allport, Profesor of Psychology, Harvard University.

I. Horizons of Psychology

The goal of psychology is the interpretation of human nature. Locke as the forerunner of present-day behaviorists and positivists (schools predominant in American psychology) postulated that the individual is born without characteristics and that his individuality is formed on the basis of life experiences. European psychology has been dominated by followers of the Leibnitzian school who believe that the individual is born with an inherent set of tendencies.

Becoming is a changing process and must take account of individual variations. Of the inborn dispositions such as instinctive species tendencies, genetic inheritance, and capacities which appear in the process of becoming (learning, conscience, self picture) these last are particularly ignored by Lockian psychologists and yet are the most important to growth. Freud does consider these capacities but on the basis of his investigations concludes that the process of becoming is essentially over by three to four years of age. This is true only in pathological conditions where growth is retarded by abnormal fixations. Normal development is not complete at that age and is free to continue the process of maturation.

II. The Process of Individualization

Although earlier scientific psychology insisted that the introduction of the concept of the soul would make psychology unscientific, more recently ego psychology has re-introduced the self. The ego may well be considered as the "style" of the individual, the proprium, that which is peculiar to each human.

Propriate aspects of personality are the center of becoming. These are eight in all: (1) bodily sense, (2) self identity, (3) ego enhancement, (4) self-extension, (5) rational activity, (6) self image, (7) propriate striving, (8) cognative self. The course of becoming is guided by propriate aspects, genetic groundwork, and opportunistic growth (adaptation and learned skills). The proprium is the monitor of behavior and evolves because the complexity of the human requires it. The time has come to recognize an oriented concept of the self and its place in becoming.

III. Oriented Becoming

Conscience is the crucial agent in becoming. The prevalent concept of conscience, the internalization of the policeman and parent, is true for early stages of growth but obviously does not hold throughout life. We discard earlier codes as we shift from inculcated habits to the proprium as the provider of values. Value schemata and their origins and determinants are now valid and important areas for experimentation.

This new psychologic orientation has much to contribute to our understanding of the religious nature of man. Religion is not (as Freud said) merely the super-ego of childhood, the fear of a punitive God. It normally develops beyond this through the efforts of the propriate aspects of personality.

The unique element in man is thus developed in his effort to relate the self meaningfully to creation. This is becoming mature.

P. B.

BEAUMONT CLUB

March 19, 1954

WATERHOUSE AND VACCINATION. By John B. Blake, Department of the History of Medicine, Yale University School of Medicine.

Dr. Benjamin Waterhouse is credited with having effected the introduction of vaccination against smallpox in America despite the great opposition of the public and the medical profession, as well as at the expense of much personal vituperation. This is attributed to a great many factors, but almost nowhere in the literature does one find any intimation that his own actions supplied a real basis for the Bostonians' opposition. In March, 1799, Waterhouse published a report on Jenner's *Inquiry* in a Boston paper. In July, 1800, he vaccinated his son. Following the report of his success, Waterhouse was deluged with requests from other physicians in the area for a supply of the "cowpox matter." Dr. Waterhouse justified his refusal to meet these requests by saying that he had developed his technique at great pains and risk to professional reputation and so wanted to keep it under his control to avoid a repetition of the early misuse of variolation in Scotland.

At about this time, Lyman Spalding of New Hampshire entered into correspondence with Waterhouse in regard to this matter. After several exchanges it developed that Waterhouse would be willing to transmit some of the "cowpox matter" to Spalding, for exclusive use in his locale, in

return for one-quarter of the profit Spalding would realize for the following fourteen months. Spalding agreed to this. The ire of the local physicians was soon aroused by Waterhouse's apparent mercenary, monopolistic attitude and success. John Warren wrote London requesting a supply of the "cowpox matter"; Samuel Brown advertised in the paper for someone who would donate matter from his vaccination "pro bono publico" and promised to distribute it to other physicians free of charge and to vaccinate without charge. He had no success.

Meanwhile, Dr. Thomas Manning of Ipswich had obtained some matter from London. This marked the beginning of the free distribution of vaccine in the United States. James Jackson, who had returned from London bringing with him only a small supply of the "cowpox matter," which failed to take, obtained some from Manning without any charge. By the middle of November many physicians had a small amount and were freely supplying colleagues. Though Waterhouse was obviously concerned with maintaining his monopoly, he also appeared to be genuinely concerned about the misuse of the vaccine. After the monopoly had been breached, he renewed his attack on the use of spurious cowpox. He was given fresh ammunition for his attack in the smallpox epidemic in Marblehead, which was attributed to the accidental use of smallpox matter for vaccine by a local physician. From this point on, Waterhouse seems to have acted on entirely different principles. He became an active agent in the distribution of vaccine in America and for nearly all of this work neither asked for nor received any money.

Waterhouse's about-face in attitude cannot excuse his self-seeking methods and tendency to self-glorification which caused him to ignore or deride those whose work with vaccinia preceded or accompanied his. When faced with the question of secret arrangements (e.g. with Spalding), Waterhouse stated that this was a method for preventing the distribution of spurious vaccine and that he did not intend to profit. His letters leave no doubt as to the nature of this fabrication.

It should be remembered that Waterhouse was the promoter, and not the originator of vaccination in America.

J. M. Q.

BIOPHYSICS SEMINAR

March 22, 1954

INFLUENCE OF TEMPERATURE AND PHASE STATES ON X-RAY SENSITIVITY OF YEAST. By T. H. Wood, University of Pennsylvania, Philadelphia.

The action of ionizing radiations on biological systems has been explained by the target theory which predicates a direct inactivation of sensitive biological units. However, since cells contain up to 80% water, irradiation-produced damage to water may be expected to play a large rôle in biological inactivation. The various kinds of breakdown products of irradiated water can diffuse to sensitive sites in the cells and act as biological "poisons." Recently, a mathematical diffusion model has been postulated to account for inactivation due to both the direct and indirect action of ionizing radia-

tions. The radiosensitivity of haploid and diploid yeasts was studied to determine the relative contribution of the direct and indirect effects of irradiation.

The percentage of survival of x-irradiated haploid yeast cells is logarithmically related to the dose, whereas the survival curve of irradiated diploid yeasts decreases only above a threshold dose. The shape of the survival curve of the irradiated offspring of their previously irradiated diploid yeast parents tends to resemble the logarithmic survival of haploid yeasts. This finding indicates that irradiation damage of one essential site of only one chromosome of diploid yeasts will permit survival, but the second generation will require only one hit of the corresponding site on the intact chromosome. Consequently, the survival curve of the second generation reveals a "memory" of the direct inactivation of the chromosomes of the irradiated parents.

The effects of irradiation can be modified by chemicals, oxygen tension, concentration of unbound water, and the kind of irradiation used. These observations provide evidence for the diffusion of poisons produced by the indirect action of irradiation. The relative biological inactivation by direct action alone was assayed by determining the difference in radiosensitivity of yeast cells and T-2 bacteriophage between the liquid and solid phases. Yeast cells and bacteriophage were supercooled in the liquid phase to —10° C. In this condition, diffusion of radiation-produced poisons in addition to direct action could cause inactivation. However, in yeast and bacteriophage frozen solid to —10° C. diffusion of poisons is inhibited. The radiosensitivity of both yeast and bacteriophage in the solid phase in which inactivation is caused only by direct action is much lower than that of yeast and bacteriophage irradiated in the liquid phase. These findings provide cogent evidence for both the direct and indirect action of ionizing radiations in biological inactivation.

ALLEN S. GOLDMAN

ANATOMY SEMINAR

March 23, 1954

ENZYMATIC STUDIES OF HAIR PIGMENTATION. By Morris Foster, Osborne Zoological Laboratory.

The amount, kind, or distribution of hair pigment in the guinea pig is controlled by six major sets of alleles, which include the yellow, agouti, albino, b (black and brown), f (reduction of yellow pigment), and p (reduction of dark pigment) series. The dark character of pigment is contributed by melanin, which results from the oxidation of tyrosine by means of tyrosinase. However, measurements of the O_2 consumption rates of guinea pig skin homogenates indicated that there is not any simple correlation between the amount or kind of natural pigment and tyrosine activity.

Our studies suggest that the rate of tyrosine utilization varies from cell to cell. This tyrosine oxidation possibly produces diffusible products, such as dopa, which can enhance the oxidation rate of slower reacting cells. This, in turn, might increase the rate of melanin formation in the formerly slower-

reacting cells, so that a chain-type reaction is established which can produce a maximum stimulatory effect. These results are analogous to those of J. R. Harrison, who found that by crowding together embryonic chick eyes in culture, an increase in their pigmentation could be produced.

P. K.

CARDIOVASCULAR STUDY UNIT

March 23, 1954

THE METABOLISM OF THE HEART. By Richard J. Bing, Professor of Experimental Medicine, Medical College of Alabama.

By combining catheterization of the coronary sinus with a modification of the nitrous oxide method first developed for measuring cerebral blood flow, both coronary blood flow and the extraction of various metabolites and oxygen by the heart muscle may be measured. The results are yielding significant information concerning the heart's metabolism.

It is found that (a) coronary blood flow, considering the vast amount of work performed by the heart, is quite small compared to that of some other organs and muscles; in the dog it is 80 cc./min./100 gm. of heart. The uptake of oxygen by the heart from its blood supply is almost maximal, 9-10 cc./min./100 gm. of heart; increased coronary blood flow, therefore, is the chief agency whereby the heart can obtain additional oxygen. (b) Carbohydrates play a minor rôle as metabolic fuels. Both glucose and lactose are only slightly utilized at ordinary, low blood sugar levels. With increasing blood sugar levels, however, there is increased utilization. Carbohydrate usage by the heart is decreased in human diabetes (though increased in alloxan diabetes of the dog). (c) Certain non-carbohydrates are more important as heart fuels, in particular fatty acids, which may be used both via the Krebs cycle and for purposes of fat storage. Amino acids and ketones are also utilized. In human diabetes, these non-carbohydrate fuel sources are made use of to a still greater extent.

The above observations would suggest that the heart obtains extra oxygen through increased coronary flow, and that it can use almost any available food stuff in the blood.

Curiously, as the heart goes into shock, it spills out pyruvate, such that the concentration in the coronary sinus is greater than that in the coronary arteries. Various mechanisms to account for this are purely speculative.

J. C. C.

MICROBIOLOGY SEMINAR

March 23, 1954

PNEUMOCOCCAL TRANSFORMATIONS AND GENETIC EXPERIMENTS. By R. D. Hotchkiss.

The stimulating work on bacterial transformation has supplied some of the strongest evidence available at present pointing to a direct relationship between highly specific desoxyribonucleic acid polymers and genetic factors responsible for the transmission of hereditary traits.

Since the initial studies of Avery, MacCleod, and McCarty on the transformation of capsular type-specific antigens in the pneumococci, several additional transformable characters have been obtained, including drug resistance and carbohydrate utilization.

The more recent work has been concerned with examining the evidence for a parallelism between the phenomenon of transformation and the classical genetic concept of inheritance. Studies on the transformation of drug resistance (penicillin and streptomycin), and carbohydrate utilization (oxidation of the sugar-alcohol mannitol), have shown the existence, in the transforming system, of phenomena analogous to genetic linkage and allelism.

In order to obtain information on the physiological nature of the transformation process in the cell, conditions were altered so as to modify the growth rate of the culture. It was subsequently found that cells underwent periodic changes in their ability to become transformed, under the influence of DNA transforming principle. These cycles, in some manner, appeared to be related to the growth and division behavior of the cell. Particularly interesting was the observation that during the period in which cells appear insensitive to transformation, DNA transforming principle was actually incorporated. However, the incorporation was only transient, in that these insensitive cells lost their newly acquired characters after a single cell division.

The important implication would seem to be that the DNA transforming principle, in order to be retained, must enter the cell at a certain stage in growth. It may be, for example, that in order to become integrated into the existing genetic material, the transforming principle must enter during a specific phase of the nuclear cycle.

S. R. SUSKIND

ARTHRITIS STUDY UNIT

April 15, 1954

THE ACTION OF SALICYLATES IN RHEUMATOID ARTHRITIS. By Edward E. Fischel, Columbia University College of Physicians and Surgeons, New York, New York.

Although salicylates were introduced seventy-five years ago, the proven clinical efficacy of aspirin in rheumatoid arthritis is difficult to explain in terms of its known biological and physico-chemical activities. These activities include antipyretic and analgesic actions, as well as an anti-inflammatory effect in certain allergy-type diseases, which, incidentally, has never been as great experimentally as clinically. Other effects, difficult to evaluate, include a protein-binding capacity, increased permeability of collodion membranes for electrolytes, lowering of the melting point of gelatin, inhibition (if given in large doses) of hyaluronidase activity, an anti-diabetic effect, increased protein catabolism, production of tinnitus in toxic doses, and increased sensitivity to CO_2 changes even in therapeutic doses. In a small study we were not able to duplicate the reported lowering of antibodies.

A pain-threshold-lowering effect is difficult to demonstrate experimentally except in the case of headache. We feel that the theory that aspirin's basic action is on the pituitary and adrenal glands has at present only tenuous evidence.

The response of rheumatoid arthritis to aspirin, although much less than that of rheumatic fever, includes relief of pain, greater motion of joints, and possibly cortisone-sparing and anti-inflammatory actions. Although definitive controlled studies are not available because of the disease characteristics and evaluatory difficulties, a recent retrospective study suggested that aspirin compared favorably with intra-articular Compound F, Corticotropin, gold therapy, and Butazole in the treatment of rheumatoid arthritis.

M. C.

ZOOLOGICAL JOURNAL CLUB

March 3, 1954

GROWTH LIMITATION AND BIOELECTRIC POTENTIALS. By Gairdner B. Moment, Department of Biology, Goucher College, Baltimore, Maryland.

Earthworms of the species *Eisenia foetida* have a definitive number of segments at hatching. The removal of segments is followed by a three-week regenerative period during which the original number, though reduced in size, is restored. Beyond this period, further proliferation does not occur, although the segments continue to increase in size. The same is true of cells in the nephrostome and gizzard-muscle. Thus, there is some mechanism which limits the final number of cells or segments.

Experiments proved the growing posterior tip of an Earthworm to be electropositive. The total Electromotive Force that an individual could generate was shown to be a function of the number of segments present, and not of their size. Tiny worms, for example, produced the identical EMF as giant worms of the same number of segments, although the amperage produced by the latter was considerably greater; (the same holds true for electric eels). The EMF would drop upon removal of segments, and its subsequent rate of increase corresponded with the rate of segment regeneration.

There appeared to be a correlation between the decline in growth rate and increase in EMF as regeneration progressed. For example, if the point where segments were removed was more distal, the effective EMF was higher and the rate of regeneration and increase in size were slower.

Child proposed a theory that explained growth in terms of dominance centers. A new Hydranth bud, according to him, would develop at the point where the shoot was sufficiently removed from the inhibiting power of a dominance center. We would suggest the reverse: that differentiation was able to occur because of an increase in the EMF to the point where further proliferation was inhibited.

P. K.

March 24, 1954

CLOCKS IN ORGANISMS. By Colin S. Pittendrigh. Department of Biology, Princeton University, New Jersey.

A truly endogenous clock is a physiological device marking absolute time that is capable of being re-set or stopped by varying the organism's metabolic rate. Bees, for example, may return to a feeding cup at a specific time each day for a period up to four days. By subjecting the bee to very high or very low temperatures, however, its clock can be accelerated or slowed, respectively, although it is temperature independent at normal ranges. This clock is of adaptive value for the bee, since the flowers exhibit periodicity in nectar and pollen production.

Several 24-hour clocks are known in which timing may be highly precise and relatively independent of the environment. These clocks represent measuring devices which arise in circumstances where the animal is interested in an event being timed. If the target time (T_t) coincides with an event in the environment which is poorly perceived and if there is a zero time (T_0) which does occur with a perceptible event, and if the organism can measure the interval from T_0 to T_t, it will make sense for it to have a clock. Indeed, it then has two clocks, one to measure 24-hour intervals ($T_0 - T_0$) and one which measures reference points within the 24-hour period ($T_0 - T_t$).

Drosophila shows cyclic activity, with the peaks occurring in the morning and evening. Eclosion is also cyclic, occurring shortly after dawn. Cultures kept in darkness show the same periodicity, so no external timing mechanism is involved in maintaining the cycle. However, experiments have indicated that the reference point for the first clock (measuring 24-hour intervals) is the dark to light transition at dawn; one such transition serving to set the clock. Flies raised in darkness from the egg stage onwards show no initial periodicity, but a single shock of light will suffice to start their cycle. The second clock which measures the interval between the dawn and evening activity peaks or between dawn and eclosion) is temperature dependent and may therefore vary. It is set with regard to a temperature based on the previous day's heat increment.

P. K.

April 14, 1954

THE NATURE OF THE TROPHIC INFLUENCE WHICH NERVES EXERT ON REGENERATION OF THE LIMBS OF THE NEWT, TRITURUS. By Marcus Singer, Department of Zoology, Cornell University, Ithaca, New York.

The regeneration of the newt limb is of interest because the process resembles the development of the embryonic limb, except that it occurs in adult tissue and under adult influences. If the amputated stump is denervated, however, differentiation and regeneration will not occur, which is not the case in the embryo.

A micro-infusion apparatus was developed enabling various solutions to be brought directly to the regenerating cells in quantities as low as .001 cc.

It was demonstrated that the use of this apparatus produced no artefacts when used for up to eighteen consecutive hours.

The infusion of water, dyes, and acids and alkalies ranging from pH 3 to 9 had no adverse effect on limb regeneration. The use of substances which normally block acetylcholine activity, however, e.g. atropine sulfate, retarded regeneration. Choline esterase, which hydrolyzes acetylcholine, also increased the delay in regeneration. From this, it can be concluded that acetylcholine may be involved in the trophic mechanism of limb growth, and a broader view of its function is therefore essential. It may be that the concept of acetylcholine as a neurohumor rather than as a specific mediator of neural impulses is more valid. If it can be proven that the trophic action of nerves is related to acetylcholine as appears to be the case, then the two functions of nerves, neural transmission and trophic action, can be shown to be one; the transmission of impulses subserving the trophic function.

Experiments involving the infusion of acetylcholine into nerveless limbs have proved inconclusive, with regeneration occurring in only one-fourth of the cases. The use of embryonic juices, as well as repetition of acetylcholine infusion, are sure to be instructive and are to be attempted shortly.

P. K.

NU SIGMA NU LECTURE

March 23, 1954

AMMONIA METABOLISM IN LIVER DISEASE AND FOLLOWING PORTAL-CAVAL SHUNTS. By William V. McDermott, Jr., Department of Surgery, Massachusetts General Hospital, Boston, Massachusetts.

The mechanism of "hepatic coma" in patients with severe liver disease and also in patients with portal-caval shunts (Eck fistulas), has long been puzzling to the medical profession. In this series, 100 patients with portal-caval shunts were studied and found to exhibit the symptoms of ataxia, confusion, mania, and even coma following the ingestion of protein, urea, or ammonium chloride. The hypothesis that these symptoms might be related to an "ammonia intoxication" syndrome was confirmed by finding markedly elevated blood ammonia levels by the Conway micro-diffusion method during periods of exacerbation.

The possible explanation is that ammonia upsets the Krebs cycle of aerobic glycolysis by drawing off α-ketoglutamic acid to form glutamic acid. Further, the administration of glutamate did permit some patients to tolerate a larger protein intake.

The recommended therapy for these patients is to control and limit protein intake, administer glutamate, and possibly to assist glycolysis by administering fructose or succinate.

P. B., JR.

BOOK REVIEWS

GENERAL BIOCHEMISTRY. By Joseph S. Fruton and Sophia Simmonds. New York, John Wiley and Sons; London, Chapman and Hall, Limited, 1953. xii + 940 pp., illus. $10.00.

This excellent textbook, as the title states, treats the subject of biochemistry from a *general* point of view, rather than from that of agriculture, medicine, or any other applied discipline. Thus the authors have drawn impartially and extensively from the vast biochemical literature on plants, animals, and micro-organisms. The book is designed primarily for graduate level courses where the student has a substantial chemical background; the authors, however, have reviewed and developed in the early chapters those aspects of physical chemistry most important to an understanding of protein behavior and enzyme activity.

In accordance with the rapid expansion of our knowledge of intermediate metabolic processes in recent years, the emphasis in this book is placed on "biochemical dynamics" rather than on structural biochemistry. This is evidenced by the seven major sections into which the book is subdivided, namely: (1) proteins, (2) enzymes, (3) biological oxidation, (4) intermediate metabolism of carbohydrates, (5) intermediate metabolism of lipids, (6) intermediate metabolism of nitrogen compounds, and (7) general aspects of metabolism.

It will be noted that the authors have departed from the more traditional order of presentation followed by most texts on the subject. In the opinion of the reviewer, however, this new arrangement is a very sound and teachable one.

After a short introductory chapter on the scope, history, and literature of biochemistry, the proteins are discussed with regard to their structure and properties and their component amino acids. The conjugated proteins, including the metallo-proteins and nucleo-proteins, are also considered. Then follow three chapters dealing with the general chemistry of enzymes, equilibria, and enzyme kinetics. Under biological oxidation, a general chapter on oxidation-reduction precedes discussion of the pyridine nucleotide-dehydrogenase systems, the flavoproteins, metallo-oxidases, and coupled enzyme-catalyzed reactions. The section on intermediate metabolism of carbohydrates includes a chapter on various methods for studying intermediate metabolism and another on the general chemistry of carbohydrates, after which consideration is given to the enzymatic formation and splitting of glycosidic bonds, anaerobic and aerobic metabolism, and photosynthesis. The next five chapters are concerned with the chemistry and metabolism of the fats and fatty acids, phospholipids, steroids, and the carotenes, terpenes, and related compounds of plant origin. In connection with the metabolism of nitrogen compounds the authors discuss the utilization of inorganic nitrogen, and various aspects of the metabolism of proteins and amino acids, porphyrins, and nucleic acids. The final section on general aspects of metabolism includes the metabolic rôle of inorganic ions, heat changes in metabolism, hormonal mechanisms, and the vitamins.

The book is rich in structural formulas and equations and contains many helpful diagrams, metabolic schemes, and tables. It is also replete with

citations of important review articles and footnotes to the more significant review literature, including many papers which appeared during the year (1952) immediately preceding publication. A substantial subject index is included. The book is remarkably free of typographical errors.

On reading this book one cannot help being impressed with the exceptional job that these authors have performed in handling such a rapidly expanding and complex field as modern biochemistry in such a comprehensive, well-integrated, interesting, and up-to-date manner. This reviewer knows of no other text to match it in its treatment of the dynamic aspects of biochemistry; consequently it can be highly recommended, not only as a text for advanced biochemistry courses, but as a valuable addition to the working library of anyone in the biological or allied sciences.

ROSS A. GORTNER, JR.

STANDARD METHODS OF CLINICAL CHEMISTRY. Vol. I, American Association of Clinical Chemists, Miriam Reimer, Editor-in-chief. New York, Academic Press Inc., 1953. xii + 142 pp. $4.50.

This is the first volume of a series that will ultimately constitute an official manual of the American Association of Clinical Chemists. It is to be published in small volumes in order that it may be more easily kept up to date by the issue, from time to time, of sectional revisions. The editorial plan is purely opportunistic. The miscellaneous methods in this volume were selected only because they are "used more frequently." In most instances only a single procedure is presented. The ostensible criteria for the selection of these procedures are precision, speed, and simplicity, with the frank statement that the first, precision, has had to be sacrificed in some instances to the secondary considerations. Each method was tested by its *Submitter* and retested by *Checkers* for "validity and practicality" before it was accepted. Although a general scheme for the presentation of procedures appears to have been outlined, the *Submitters* have evidently been given considerable freedom in their interpretations of this outline.

Urgency is a matter of opinion. Others besides the reviewer may wonder why the particular miscellaneous methods in the first volume were given precedence over others. Even if the selection on the basis of urgency met with universal approval, the adoption of this policy must necessarily compromise broader principles which should prevail in such a handbook. The volumes are evidently intended for persons of the technician grade. The discussions of the principles and significance of the tests vary greatly in scope and length but are in general quite inadequate for those who really desire expert information. The bibliographies are equally deficient. Some chapters have none; in others they are irrelevant. The reader is not, therefore, tempted or aided to delve further into any of the topics discussed. Neither the reasons for the selection of certain procedures nor references to other reliable techniques are regularly supplied. In other respects the length and format of the chapters are not consistent. The differences do not seem to be related to the importance or complexity of the procedures, but to the

caprices of the individual authors. The descriptions of the methods are brief, but again whimsically variable in style and arrangement. For example, on pages 56 and 57 identical *stock standards* for creatinine are described in entirely different terms. With respect to methods of calculation the text is equally inconsistent. The sections dealing with precision of procedures and normal values are uncritical and purely emiprical. "Checking" is open to criticism, especially when the methods and subjects used are heterogeneous. These sections occupy space out of proportion to their value. The table on page 41, for example, deserves no place in such a manual. The limits, "99-111 mEq. chloride per liter serum," on page 39 of the same chapter are sharply at variance with those cited by reliable observers derived by more precise methods.

If variety in matters of this kind in a compendium is justifiable or desirable, precision must never be sacrificed by the editors. On page 56, under Procedure, "Measure 5 ml. of the serum and blank centrifugate into two photometer cuvettes," if not unintelligible in its context, is at least confusing. The statement on page 36 that serum CO_2 is increased in hyperventilation, if not a missprint, is an egregious error. These are not the only errors of their kind, but only examples of types of errors that are found in the volume.

Two procedures instead of one are given for serum bicarbonate, blood glucose, and blood creatinine. Since those for bicarbonate involve entirely different principles, the departure from established policy is comprehensible. The Folin-Wu blood sugar method, which is admitted to be highly nonspecific, seems to have been included for purely sentimental reasons. No good reason is offered for presenting two methods for creatinine. If the editors are convinced that the dinitrobenzoic method is more specific since it agrees with the alkaline picrate method if absorption with Lloyd's reagent is included in the latter, it would have been logical to present either the dinitrobenzoic method or the alkaline picrate method with the Lloyd's reagent step. The Schales' procedure for chloride was a poor selection on more accounts than one. It cannot be applied directly to serum and, as the author admits, the use of filtrates introduces a variable systematic error. It also gives erroneous results when applied to urines that are not fresh (p. 42); to this should be added those that are heavily albuminous. There are excellent and relatively simple methods that can be applied directly to serum and urine that are not subject to these errors. There are distinct disadvantages to using filtrates. Procedures for the measurement of other electrolytes are usually applied to serum directly. Since measurement of chloride is chiefly useful as one of the component parts of the estimation of electrolyte or acid-base equilibrium, accuracy, convenience, and economy of material are served if the serum can be treated in the same manner for all the procedures.

The presentation of procedures in the opportunist manner described above does not provide for the discussion of general principles nor for adequate consideration of the suitability of each of the miscellaneous methods to the others that should ultimately make up the completed system, and particularly those with which it can be combined for chemical or clinical

convenience. Those who will need or desire such a narrowly defined system as this manual will offer when complete will also require more background of simple chemical principles than this plan will afford them. The volume does not offer such fundamental material. There is a seven-page introduction that deals with laboratory amenities in such a cursory manner that the reader could not possibly carry out the procedures discussed without other help. The last two pages are devoted to altogether inadequate remarks about the use and care of photometers although these instruments are used for the majority of the procedures in the volume. In these procedures all specific directions apply to the Coleman spectrophotometer. A carefully designed chapter on the principles and use of this instrument, if standardization is to be carried that far, in detailed terms, with appropriate illustrations, would greatly enhance the value of the volume and save space in the descriptions of the procedures in which it is used. Eight pages of the carbon dioxide chapter are devoted to the original volumetric apparatus described by Van Slyke in 1914, only five pages to the manometric apparatus which has long since superseded it. If the whole thirteen pages were given over to the description of the manometric apparatus, it is doubtful whether a technician could master the procedure without other aid. The Van Slyke manometric apparatus can be employed for a great many purposes and is especially adapted to occasional determinations because the gasometric procedures require few, if any, accurate quantitative reagents. An adequate description of the apparatus with a detailed discussion of its operation would, therefore, be of great value, especially if it appeared as a preface to all the gasometric procedures that may be presented and contained references to other applications of the apparatus. It is becoming more and more evident that cholesterol cannot serve as a symbol for all lipids; but, if a more extensive partition of serum lipids is contemplated, the method adopted for cholesterol should be adapted to such a partition. The lipid methods should be prefaced by a discussion of techniques for the analytical treatment of lipoid material. The chapter on flame photometry apprises the readers that there is an apparatus available. It is not only inadequate, but also inaccurate. The statement, "sodium and potassium give off good emission spectra even with city gas" (p. 103), is illusory. The Barclay instrument, which the author uses for illustrative purposes, is now equipped or can be equipped with a closed atomizer (see p. 105). The bibliography is paltry. As the author says in the next to last sentence in the chapter, "A more or less explicit set of directions comes with each apparatus," but this does not aid in the selection of the most suitable apparatus.

The indices are a waste of space. The author index is a clumsy guide to names in bibliographical references; the subject index is merely a repetition of the table of contents with section headings.

The fact that this set of handbooks is intended to so large an extent for the guidance of persons lacking great expertness and a broad background of fundamental chemistry makes it the more imperative that it be meticulously explicit and precise. Since it purports to set up a system of analysis for clinical chemistry, the methods should be selected with an eye to coordination and organization. Precision cannot be sacrificed to convenience.

The fallacy of the popular concept that "it's good enough for clinical purposes" is becoming more widely recognized as the potentialities of scientific methods in diagnosis and therapy increase. Such a manual as this should aim to raise the dignity and improve the quality of clinical chemistry and clinical chemists, not, by conceding to prevailing opinion, perpetuate its present mediocre estate. Rigid standardization by selection of single methods may not be the best policy. The fact that the series is to appear in small consignments affords opportunity for improvement with progress. It is to be hoped that the editors will profit from the errors in their trial balloon.

JOHN P. PETERS

PHYSICAL CHEMISTRY FOR STUDENTS OF BIOLOGY AND MEDICINE. 4th Ed. By David Ingersoll Hitchcock. Boston, Little, Brown and Company, 1953. 266 pp. $5.00.

This book is written for use in a "short course." Its format is essentially the same as that of previous editions. Each chapter is followed by a set of problems, and at the end of the book there is presented a carefully selected series of fourteen laboratory experiments. The subject matter is presented simply. The mathematics employed is limited almost entirely to elementary algebra. The author's purpose has been to discuss briefly a few of those principles of physical chemistry that have found widest applicability in the work of the biological scientist.

Professor Hitchcock is to be congratulated warmly on the very lucid and accurate manner in which he presents his material. To write well a short discussion of a complex subject is not an easy assignment. Only too often the student of an abbreviated treatment later finds himself faced with the necessity of unlearning things incorrectly learned. The reader of Professor Hitchcock's book will have no such problem. The reviewer recommends it most highly to those who want a succinct, clear treatment of some of the fundamentals of physical chemistry.

It is regrettable that in the current edition there is not included the preface to the first edition, for this preface would do much to help the reader in placing the text in proper perspective. No attempt is made to cover all those subjects that might be of interest to students of the biological sciences. Indeed, the discussion is limited largely to a consideration of the behavior of dilute aqueous solutions, with special emphasis on solutions of electrolytes. This is done in the belief that a knowledge of this material should precede the study of other aspects of physical chemistry.

It is obvious that in a book as brief as this one, there must be a very strict selection of material. It is also obvious that Professor Hitchcock has made a very admirable effort to keep the size of his book from increasing with the passing editions. Students would profit, however, from discussion of a number of subjects which have not been touched upon, and this reviewer, for one, feels that the advantages of a limited expansion would outweigh the disadvantages. He hopes that Professor Hitchcock will consider applying his abilities of exposition to a wider range of subject matter in the next edition.

The reviewer hopes that the reader will forgive the following personal observation. No "short course," however well presented, can give a student the training that a good year-long course can give. No one is more aware of this than is Professor Hitchcock, as the preface to the first edition will testify. The reviewer is disturbed by the fact that the majority of biological scientists, if they take any training in physical chemistry at all, limit such training to a short course. This is today no more adequate than would be, for example, a short course in histology or physiology. The serious student of biology or medicine should be urged to find time in his schedule for a year's course in physical chemistry as it is usually presented in departments of chemistry.

HENRY A. HARBURY

HISTOLOGY. 2d Ed. By Arthur Worth Ham. Philadelphia, J. B. Lippincott Co., 1953. xix + 866 pp. $10.

Within the pages of any book as comprehensive as Dr. Ham's *Histology* it is rarely difficult to find at least a few points meriting criticism. These may include such relatively minor items as the omission of the primary verb from a sentence (p. 283, column II), or more serious charges of vagueness in description (p. 328, contraction phenomena). Yet, the overall effect is a good one. The outline of the subject material remains basically unaltered from the first edition, though valuable additions, particularly in the area of muscle physiology and chromosomal behaviour, have been made. With selections concerned with general histologic techniques, detailed considerations of the different tissues and of the major systems, this book will continue to rank as a thoroughly readable and reliable histology text. This quality of readability, however, while doubtless ly endearing the text to medical students, will prove somewhat of a handicap to those in need of a purely reference volume. Particularly Dr. Ham's predilection for analogies will prove irksome to those searching for a concise compendium, while simultaneously evoking cheers from students with no love for histologic-monotonies. The extensive and well-organized bibliographies, however, will be a source of joy to either of these readers.

P. K.

BAILEY'S TEXT-BOOK OF HISTOLOGY. 13th ed. Revised by Philip E. Smith and Wilfred M. Copenhaver. Baltimore, The Williams and Wilkins Co., 1953. xviii + 775 pp., 442 figs., $9.00.

The author of an elementary textbook is confronted with a choice between presenting his material in a straightforward, factual manner and writing a jocular, quasi-factual account. The first alternative makes for accurate knowledge and dull reading, whereas the second makes for confused thinking and entertaining reading. The authors of the text herein reviewed chose the first alternative. There must be an intermediate state which emphasizes the conceptual scheme of a subject into which the factual material can be fitted naturally without distortion, and which at the same

time is clear, coherent, and a pleasure to read. Such a text has not yet appeared in histology.

The first edition of Bailey's textbook appeared in 1904 and has been revised assiduously since then. It is probably a mistake on the part of the publishers to issue repeated revisions of their standard texts. The resultant inertia in favor of retaining most of the illustrations and large segments of the text, with as little change as possible throughout, restricts the editors or authors to the form, if not the substance, of the previous editions, and the complete rewriting which is often advisable must be abandoned in favor of editing. Such a result is seen in this text, in which about half of the illustrations are old charcoal drawings that contrast sharply with the fine modern photographs and wash drawings alongside them. The few color plates are almost all poorly reproduced as the different colors are usually not in register. In some places the illustrations have not kept pace with the revisions of the text. It is difficult to understand, for instance, what purpose is served by the poorly reproduced color plate of Vierling's reconstruction of the liver (Fig. 278) which is based upon an outmoded concept of hepatic structure, or by Figures 402 and 403, which are not self-explanatory and to which no reference is made in the text. Only three electron micrographs, although of superb quality, have been included in this book, and little indication is given of the profound contribution which electron microscopy has already made to the advance of morphological science. Generally, however, the text has been revised to include the advances in microscopic analysis of tissues, such as the structure of the liver and the neurohypophysis, and the histochemistry of the intercellular ground substance. In some places, however, the text has been revised without bringing it up to date, as in the discussion of the adeno-hypophysial control of lactation and in the discussion of renal histo-physiology. Nevertheless, this book may be considered as an adequate text for first-year medical and dental students, although one should not be considered captious for remarking that a really first-class presentation of this subject remains to be written.

SANFORD L. PALAY

BASIC PATHOLOGY AND MORBID HISTOLOGY. By D. B. Cater. Baltimore, The Williams and Wilkins Co., 1953. 320 pp., 263 figures.

Conceived in the years of Dr. Cater's experience as demonstrator and lecturer in pathology at Cambridge and as Head of the Surgical Unit, Lester Chinese Hospital, Shanghai, and dedicated to the proposition that "our minds can only understand new ideas in terms of what is already clearly grasped," this book is an exceedingly welcome addition to that limited group of pathology texts from which a student may profitably choose. With a proper regard for the essential rôle of stimulating rhetoric and an occasional dart of humor, and by addressing himself primarily to a reader who would prefer an elucidation of fundamental principles to being beleaguered by a horde of facts, the writer maintains traditional British pride of authorship much as does Dr. William Boyd in his text on pathology.

This work is divided into three major sections. The first, entitled "The Reaction of the Body to Infection," stresses the dynamic concept of the host's reaction to an invasion of organisms as "a battle full of surprises: both sides are able to alter tactics"; special chapters are devoted to syphilis, tuberculosis, and typhoid fever. The second, "Diseases Due to Breakdown of Physiological Mechanisms," is of great value for its unbiased view of arteriosclerosis and a comprehensible discussion of the sequence of events following various types of heart pathology, while the third, "Tumours," contains a particularly challenging chapter on the possible aetiology of cancer as seen in the light of experimental carcinogenesis.

The avowed purpose of this book is to make pathology intelligible to the aspiring student; its excellent camera lucida drawings contribute no small part to this end. Dr. Cater attempts to present a work which will disperse the all too prevalent impression that "pathology is a science of dead things —a static subject; a science whose high altar is the marble slab of the post-mortem room; a science whose high devotees haunt dusty museums, gazing at bottled specimens." This reviewer would respectfully submit that the author has admirably attained his goal.

J. C. C.

DISEASES OF THE RETINA. 2d Ed. By Herman Elwyn. New York, The Blakiston Co., Inc., 1953. xiv + 713 pp. $12.00.

The second edition of Elwyn's excellent book, *Diseases of the Retina,* follows in general the text of the first edition. The chapter on Diabetic Changes in the Retina has been enlarged and several illustrations added. Expansion of the chapter on Heredodegenerative Changes and that on The Clinical Picture of Retinitis Pigmentosa gives more information on these subjects.

A chapter on tuberculosis, covering allergy and immunity in general, has been added. Two other new chapters have been introduced, that on Retrolental Fibroplasia and one on Sarcoidosis of the Retina. The new illustrations on Disciform Degeneration of the Macula, Amaurotic Family Idiocy, and Gyrate Atrophy of the Retina increase the value of the text.

A general lengthening of the bibliography brings the book up to date for reference, and this fact, together with the improved text, makes this fascinating study of the innermost layer of the eye most valuable. "Elwyn" is a book needed by every ophthalmologist.

EUGENE M. BLAKE

THE EFFECT OF ACTH AND CORTISONE UPON INFECTION AND RESISTANCE. Gregory Shwartzman, Ed. New York Academy of Medicine, Section on Microbiology, Symposium No. 6, March 27-28, 1952. New York, Columbia University Press, 1953. v + 204 pp. $5.50.

This book is a collection of thirteen papers presented at a meeting of the Section on Microbiology of the New York Academy of Medicine nearly two years ago. Although most of the articles deal with particular phases of

immunity or infection as influenced by adrenal steroids, some describe biochemical or anatomical studies that are only indirectly related to the subject of the symposium. Each article is by an acknowledged authority; most consist of a review of published reports up to the time of the meeting. Liberally interspersed are interpretations and criticisms, often on the basis of the investigative findings of the writer himself, and the summaries usually achieve a point of view or approach to the particular problem which is more valuable than the assorted facts alone could ever be.

The introductory chapter on "Some General Considerations Concerning the Role of the Adrenal Cortex in Intermediary Metabolism" by Frank Engel is a superb discussion of the importance of endocrine interrelationships in maintaining homeostasis; he cautions immunologists, bacteriologists, and virologists against the fallacy of equating the effects of ACTH or cortisone and "normal" adrenal function, emphasizing further the necessity of defining "normal" endocrine function in terms of internal and external environment at the time measurements are made. Citing classic examples of previous error resulting from failure to recognize that biochemical or metabolic changes after ablation of a single gland or injection of a single hormone cannot be interpreted solely in terms of the hormone in question, Engel warns that the Law of Parsimony usually does not apply in interpreting the results of any experiment which involves alteration in the delicate endocrine balance of the entire organism. Lastly, he pleads for wider recognition of so-called "permissive" hormone action, a concept which has been so beautifully illustrated in his own and in Ingle's classic experiments on the metabolic alterations produced by stress.

The appearance of these papers in print nearly two years after their delivery might lead one to fear that the material would be outdated. However, the results of so many "personal communications," "unpublished results," or "preliminary experiments" were described at the symposium that the details of much of the work mentioned have been published in medical journals only recently and some are still unpublished. Furthermore, the basic concepts and suggestions of pathways for further investigations that are contained or implied in this symposium at least equal and will eventually far exceed in value the factual content.

IVAN L. BENNETT, JR.

BIOCHEMICAL PREPARATIONS, Vol. 3. Esmond E. Snell, Ed. New York, John Wiley and Sons, Inc., 1953. viii + 128 pp. $3.50.

Volume 3 of this series contains descriptions of the preparation of the following substances: crystalline muscle phosphorylase (B. Illingworth and G. T. Cori); ribonuclease (M. Kunitz); diphosphopyridine nucleotide (A. Kornberg and W. E. Pricer, Jr.); triphosphopyridine nucleotide (A. Kornberg and H. L. Horecker); pyridoxamine phosphate and pyridoxal phosphate (E. A. Peterson, H. A. Sober, and A. Meister); protoporphyrin dimethyl ester (V. G. Ramsey); d-isocitric acid (H. B. Vickery); dl-isocitric acid lactone (H. P. Kato and S. R. Dickman); dihydroxyfumaric

acid (E. F. Hartree); oxaloacetic acid (C. Heidelberger); L- and D- lactic acids (M. Brin); sodium alpha-ketoisocaproate (A. Meister); D-glucose-6-phosphate (W. A. Wood); D-galacturonic acid (K. P. Link); beta-2,5-dihydroxy-phenyl-DL-alanine (H .B. Gillespie); L-isoleucine, D-isoleucine, L-alloisoleucine, D-alloisoleucine (J. P. Greenstein, S. M. Birnbaum, and Leon Levintow); DL-ornithine monohydrochloride, L- and DL- citrulline (from ornithine - P. B. Hamilton and R. A. Anderson); L-ornithine monohydrochloride (D. E. Rivard); L-citrulline (from L-arginine - V. A. Knivett); L-kynurenine (Osamu Hayaishi); L- and D- pencillamine hydrochlorides (B. E. Leach and J. H. Hunter); elaidic acid (D. Swern and J. T. Scanlan); 2,4-dinitrofluorobenzene (G. C. Finger and J. L. Finnerty).

As in the previous volumes, the reliability of the procedures and the adequacy of the directions were established by having each method checked in a laboratory other than that of the submitter. The clarity of the instructions as well as the great detail with which they are given is impressive, especially when compared with the highly condensed manner in which directions often appear in the literature. In general, procedures have been brought up to date. For example, a comparison of several procedures in the literature with their treatment in this volume indicates that many had undergone modification. Although the preparation of diphosphopyridine nucleotide appears in Volume 1, a superior modification (chromatography) appears in Volume 3. It is factors such as these which raise the value of *Biochemical Preparations* to a level much above that of a mere collection of methods.

Although the contents of Volume 3 are of wide interest, the series in general could be made a great deal more valuable by increasing the proportions of enzyme preparations, especially of those enzymes which are extensively used as reagents, e.g. alcohol dehydrogenase, hexokinase, etc. In the enzyme preparations given in the series, it is regrettable that, with the exception of phosphorylase, no mention is made of the specific activities at maximum purity. The manner in which phosphorylase was written up can well serve as a model for all other enzyme preparations. Notable also is the lack of peptide preparations in the series. With the exception of glutathione and its intermediates, none appear.

In addition to methodological detail, each preparation contains a description of the starting substances, which is especially important in procedures based on isolations from biological materials. Also included are discussions of the properties and purity of the products with references and outlines of the methods of determining purity. A final selection lists other methods of preparation. The volume also includes a cumulative index covering the entire series and a list of compounds of biochemical interest which have appeared in *Organic Syntheses*.

How valuable this particular volume will be to any given individual depends upon his specific biochemical interests. In this connection, its value for teaching purposes should not be underestimated. Almost any of the procedures could be introduced into a graduate laboratory course without further testing.

As a series, these volumes give promise of filling a place in the biochemical literature similar to that held by *Organic Syntheses* in the field of organic chemistry.

MELVIN SIMPSON

CONNECTIVE TISSUES. Charles Ragan, Ed. Transactions of the Fourth Conference. February 18-20, 1953. New York, Josiah Macy, Jr. Foundation, 1953. 197 pp. $3.75.

A conference on connective tissues is sponsored yearly by the Josiah Macy Foundation. Its purpose is to initiate an exchange of ideas among workers interested in the problem and who are drawn from varied disciplines, i.e., biochemistry, pathology, clinical medicine, pharmacology, etc. The transactions are published as verbatim accounts of the informal discussion which takes place during the presentation of papers. This is done to give a realistic and unprejudiced picture of the type of thinking that is being applied to solve problems in this field. The conference was attended by members Charles Ragan, D. Murray Angevine, Granville A. Bennett, Edward W. Dempsey, Karl Meyer, Keith R. Porter, Janet Travell, and Benjamin W. Zweifach, with W. Paul Holbrook acting as chairman. One guest, George M. Hass, reported on the "Isolation and Characterization of Mammalian Striated Myofibrils," while another, Honor B. Fell, in a much shorter session reported on her work with Sir Edward Mellanby concerning "The Effect of Vitamin A on Organ Cultures of Skeletal and Other Tissues." Both of these papers are well illustrated. An introduction, introductory remarks, a general discussion on supposed areas of agreement reached in this conference, and a discussion of problems to be solved are also included.

WILLIAM G. BANFIELD

CYBERNETICS: CIRCULAR CAUSAL AND FEEDBACK MECHANISMS IN BIOLOGICAL AND SOCIAL SYSTEMS. Heinz von Foerster, Ed. New York, Josiah Macy, Jr. Foundation, 1952. xx + 240 pp. $4.00.

This volume represents the Transactions of the Eighth Conference on Cybernetics that took place in 1951. Dr. Frank Fremont-Smith, speaking for the Josiah Macy, Jr. Foundation which sponsors these conferences, makes it clear in his introduction that "our meetings are in contrast to the usual scientific gatherings. They are not designed to present neat solutions to tidy problems but to elicit provocative discussion of the difficulties which are being encountered in research and practice. For this reason, we ask that the presentations be relatively brief and that emphasis be placed on discussion as the heart of the meeting." Finally, he concludes, "By preserving the informality of our conferences in the published transactions, we hope to give a truer picture of what actually goes on in the minds of scientists and of the role which creativity plays."

This volume faithfully adheres to all these aims. The reader is taken into the bedroom of men's minds and is exposed to all which that entails. For

all the humdrum of mental undressing, dressing, shaving, etc., there is the chance reward of being confidential ear to what may never be so well expressed or thought of again. The contributors of the "relatively brief" presentations are six in number. Alex Bavelas talks on "Communication patterns in problem-solving groups"; Ivor A. Richards, on "Communication between men: Meaning and language"; Lawrence S. Kubie, on "Communication between sane and insane: Hypnosis"; Herbert G. Birch, on "Communication between animals." Claude A. Shannon gives a presentation of a maze-solving machine, and Donald M. Mackay concludes with a paper called, "In search of basic symbols."

All the speakers are interrupted so frequently by questions, commentaries, and arguments of the discussants that their subject matter never seems to gather sufficient momentum "to take off." For the methodical reader who likes to keep the story straight, these constant, and not always relevant, interruptions may generate some degree of hostility towards the discussants. But this need not lead to undue anxiety and an eventual nervous breakdown, for he has either the choice of laying the book down, or relaxing in the phantasy that he is observing an intellectual game of fox and geese. In addition, he may reassure himself that he is not peculiarly alone in his annoyance, because MacKay, speaking last, and apparently having done a good deal of anticipatory fretting on this score, begins his presentation by saying, "I don't know whether you people feel agreeable, but if there are any questions which you believe are not absolutely vital . . . I should be very grateful if you would make a note of them and bring them all up at one time rather than interrupt the flow. . . ."

By and large, however, the foregoing objections will not be disturbing to the person who is able to read fast and to scan over the trivia. Nor do most of the discussions merit the comment of the wag who said the subtitle of this conference should have read, "Circular causal mechanisms, or arguing in circles." During the summer when this book lay on a table with an assortment of other reading material, I saw it singled out and enjoyed by both lay and professional readers alike. There is no doubt that much of it represents an interesting discussion of interesting subjects by interesting minds. In addition to the transactions it includes an excellent editors' note by von Foerster, Mead, and Teuber on the scope and aims of the cybernetic conferences, as well as an appendix by Mackay which will be most helpful to those who are bewildered by, but wish to familiarize themselves with, the nomenclature of information theory.

PAUL D. MAC LEAN

ADAPTATION IN MICRO-ORGANISMS. The Third Symposium of the Society for General Microbiology. E. F. Gale and R. Davies, Eds. New York, Cambridge University Press, 1953. ix + 339 pp. $6.00.

The Society for General Microbiology held its third symposium in London on April 14 and 15, 1953. *Adaptation in Micro-organisms* is the published record of this meeting, consisting of fifteen contributions and an edited report of the discussion of each contribution. The contributed papers

are in general excellent, and the printed discussion is both informative and interesting. This volume reviews the field very well and indicates aspects of the field in which there is diversity of opinion.

This volume is mainly concerned with problems of bacterial adaptation and considers many different aspects, including the problems of genetic and non-genetic factors in adaptation, enzymatic adaptation, and drug resistance. However, certain aspects of adaptation in protozoa and fungi are also considered. Enzymatic adaptation is perhaps the most thoroughly discussed aspect of adaptation, with a group of fine papers by S. Spiegelman, H. A. Halvorsen, M. Cohn, J. Monod, M. R. Pollack, P. P. Slonimski, and R. Knox. These papers, together with the discussions, cover the known facts of the field and go on to add a good deal of interesting speculation on the induction process.

The many problems of drug and antibiotic resistance are covered in a series of papers by E. P. Abraham, Mary Barber, D. A. Mitchison, and L. F. Hewitt. Adaptations in Paramecium are discussed by G. H. Beale, in fungi by W. Brown and R. K. S. Wood, and in thermophiles by L. F. L. Clegg and S. E. Jacobs. The influence of bacteriophage on bacterial variation and evolution is considered in a fine paper by L. F. Hewitt.

Throughout the volume, the controversy over genetic factors versus non-genetic factors in adaptation plays a prominent rôle. Two papers, one by Sir Cyril Hinshelwood and the second by A. W. Ravin, specifically consider this problem. There can be no doubt that gene mutation and selection account for a majority of adaptations of the drug resistance type. The possibility of other types of adaptation, however, is widely discussed. The general discussion of this problem is well done and clarifies many of the problems that confront workers in this field.

The volume is well printed and well edited and is heartily recommended to anyone interested in the problems of microbial adaptation.

DAVID M. BONNER

THE PSYCHIATRIST, HIS TRAINING AND DEVELOPMENT. Report of the 1952 Conference on Psychiatric Education held at Cornell University, Ithaca, New York, June 19-25, 1952. Washington, American Psychiatric Association, 1953. viii + 214 pp.

This book is a very well written and interesting account of the general features of postgraduate psychiatric training in the United States today. It begins with a brief historical review of the development of the residency system, goes on to discuss prevalent feeling as to the rôle of psychodynamics, psychoanalysis, and child psychiatry in psychiatric training. Because some of these topics (i.e., psychoanalysis) in their very nature are controversial, even in the field of professional psychiatry today, the conclusions reached are often nonspecific, but even these are recorded in such a way as to give information concerning the major points of agreement and disagreement among the participating authors. Psychiatric training in relation to other medical specialties, including public health, and the implications this has for training programs are considered at length. A large sec-

tion of the conference report is concerned with considerations more closely related to the resident as an individual. It considers the type of life he leads, his choice of psychiatry as a profession, the selection of residents, the training centers, and the ideals and realities of training programs. In these chapters there is greater agreement resulting in more specific and to-the-point discussion of the topics.

I would highly recommend the book to any student or teacher interested in the field of psychiatry, and the greater part of it to anyone interested in finding out what psychiatry is today and hopes to be in the future.

P. B.

GUIDE TO THE CLASSIFICATION AND IDENTIFICATION OF THE ACTINOMYCETES AND THEIR ANTIBIOTICS. By Selman A. Waksman and Hubert A. Lechevalier. Baltimore, The Williams and Wilkins Co., 1953. x + 246 pp. $5.00.

This is a book that will have an important place on the library shelf of the soil microbiologist and the medical mycologist. It is not a text but rather a classification of the actinomycetes and their antibiotics. The volume is divided into two sections: Part I, Classification of the actinomycetes, and Part II, The antibiotics. To the neophyte, this book will be difficult to use, but to those who every day must isolate and identify species of actinomycetes it is most valuable. For the medical mycologist it represents the first key available for the differentiation of the Streptomyces, which usually are considered non-pathogenic for man, from the Nocardia, a genus containing species pathogenic for man and animal. The key offers a means of identifying the known species of Nocardia which is always a most difficult job for the medical mycologist. Part II, dealing with the groups of antibiotics, consists of two keys. Key 1 primarily describes the antibiotics through their chemical and physical properties; key 11 describes the antibiotics on the basis of activity against micro-organisms, although certain chemical properties are also included.

The final section of this book includes a list of the antibiotics isolated with a brief discussion of their source, method of isolation, chemical and physical properties, biochemical activity, toxicity, and utilization.

It is to be hoped that this work did not exhaust the authors for even as this review is written, new strains of actinomycetes and new antibiotics are being isolated, all of which will necessitate occasional revision of this excellent volume.

LEANOR D. HALEY

SCIENCE AND MAN'S BEHAVIOR. By Trigant Burrow. New York, Philosophical Library, 1953. xii + 564 pp. $6.00.

This posthumous presentation of the author's final work can be characterized as reflections in phylobiology. Starting with the problems of the United Nations Organization, he traces the origin of the cataclysmic crises of our time to the intrapersonal difficulties of man as a phylum. The discussion of the world's problems are at a journalistic level deliberately avoid-

ing the sophistication of anthropological, sociological, and political analysis. A theory of psychology is proposed which is strongly ethical in implication. The psychic functions supporting self-gain and the offense of others are viewed as the ingredients of the social neurosis which breeds war and atomic destruction. He defines affect in the individual as an unwarranted feeling based on wishful self-interest or self-bias. A similar quality of disorder is traced through perception and prejudice to a neurophysiological substratum. The discussion of the biological foundation underlying all social phenomena is highly metaphorical. Also the experimental evidence presented in an appendix is very scanty in terms of the magnitude of the theory it is intended to support.

The principal text is preceded by approximately a hundred pages of criticism and refutation taken from correspondence with an impressive group of scholars who had received a prepublication edition. It is apparent that the author believed that isolation from the mainstream of modern psychiatric theory was necessary for his creative efforts. Even the list of eminent critics reflects this isolation. His terminology is unique but thoroughly defined amid many examples which tend to be repetitive. The psychotherapist and social actionist will find little encouragement in this assessment of the evolutionary and organic imperfection of man as a species.

BERTRAM H. ROBERTS

ADVANCES IN VIRUS RESEARCH. Vol. I. Max A. Lauffer and Kenneth M. Smith, Eds. New York, Academic Press, Inc., 1953. vii + 362 pp. $8.00.

This volume, the first of a promised series, should be read by all who are interested in the viruses. Virus infections are common in many forms of life, and probably occur in others where they have not as yet been recognized. But the worker in any one field is put to it to keep informed of the advances in his own area, so that this series should have a warm welcome.

In this first volume the editors have chosen to include the following topics: The Properties of Bacteriophages, Interactions between Viruses and Virus Strains, Transmission of Plant Viruses by Cicadellids, Insect Viruses, Multiplication of Influenza Virus in the Entodermal Cells of the Allantois of the Chick Embryo, Poliomyelitis, Purification and Properties of Animal Viruses, and Virus Nucleic Acids.

To this reviewer (because he is more acquainted with these fields?) the articles on Influenza and on Poliomyelitis seemed exceptionally complete, but all were read with pleasure and profit. Perhaps the section on the bacteriophages could have been expanded with advantage, since the study of these viruses is yielding so many clues to the general behavior of others. The author's decision to curtail his discussion of lysogenesis is to be regretted also.

In reading the reviews of virus infections in plants and in insects, the animal or the bacterial virologist will constantly be reminded of the many similarities between the patterns with which he deals and those found in other phyla. And in the last two sections, the reader will be brought up to

date on the properties of those animal viruses which have been most thoroughly studied and on the methods used in these studies, and will read an informative discussion of the nucleic acids, which make up so important a fraction of the virus particle.

Errors are few, but it is refreshing to read about "V. cohlerae," and to learn that phages can be "eluded"!

P. B. COWLES

DIE ERNAHRUNG. PHYSIOLOGIE, PATHOLOGIE, THERAPIE. By Konrad Lang and Rudolph Schoen. Berlin, Springer-Verlag, 1952. vii + 624 pp.

This book is divided into fourteen chapters, each written by a separate author. The titles are: Food Chemistry, Physiology of Nutrition, Food Technology, Malnutrition and Overeating, Dietetics, Physiology and Pathology of Child Nutrition, Nutrition of Population, Physiology of Vitamins, Symptoms and Therapy of A-avitaminosis, Symptoms and Therapy of E-avitaminosis, B-vitamins, Vitamin C, Rickets, Tetany, and Osteomalacia, Vitamin K.

The two introductory chapters on Food Chemistry and Physiology of Nutrition give rather general information, the latter going beyond the limit of physiology and describing, for instance, the importance of particular foods such as milk and bread for good nutrition. The high oxalic content of cocoa (0.45-0.48% is mentioned.

The chapters on malnutrition and vitamin deficiencies are highly specialized; they are based on clinical work and deal with the therapeutic applications of vitamins. The studies of malnutrition were made on prisoners of war returning home from Russia, and the author remarks that the degree of severity of starvation symptoms increased with the height and age of the subjects.

The chapters on the vitamin B complex, vitamin C, and vitamin K are of a general type, describing the signs of deficiency and probable physiological rôle of the vitamins in question. The chapters on Food Technology and Dietetics represent a technical approach to the problems, the first one describing the advantages of various food processing techniques, the other evaluating a variety of diets in terms of their nutritive content.

Because the contributions vary in scientific "level," the book as a whole gives the impression of lacking homogeneity, but as a collection of useful information and as a source of German literature on nutrition, it is a valuable supplement to a library of nutrition.

W. A. KREHL

THE AUDIOLOGY CLINIC. A MANUAL FOR PLANNING A CLINIC FOR THE REHABILITATION OF THE ACOUSTICALLY HANDICAPPED. By Moe Bergman, Ed. Acta-Oto-laryngologica, 1950. Supplementum 89. $1.00.

After extensive experience during the war with the Rehabilitation of the deafened in the Army Training Center at Deshon, Pennsylvania, the author was inspired to continue his work in a civilian clinic. Following time spent

in Washington, D. C. in connection with the central office of the Veterans Admnistration, he was appointed to the staff of the Audiology Center of the New York Regional Office. The Rehabilitation Center which Bergman describes in much detail in the manual was developed at that regional office. Approximately 6.500 square feet of space was allotted for the purpose of handling the acoustically handicapped.

With extraordinary enthusiasm the author not only was responsible for the educational program of a large number of veterans, but he saw fit to continue with his own education. He enrolled in Columbia University and obtained his Doctor of Education degree while working at the Veterans Center.

Rehabilitation of the deafened is a multi-phasic program and in the Bergman monograph every phase is considered. The scope of the activities of the clinic are indicated from the beginning with a medical examination, including refer for otologic treatment if there is any possibility of recovery of any portion of the hearing loss, and continuing through to the placing of veterans in proper positions. Hearing evaluation, hearing aid selection, auditory training, lip reading exercises, and psychological adjustment are all included.

The time needed for proper rehabilitation varies with different individuals. The clinic has provided two main types of program. The first one requires two and one-half days and is an orientation course. It assists in the selection of a hearing aid and introduces the patient to the complete program which will be of value to him later. The second program requires four weeks and is given at a time when the veteran can arrange to be away from his work. Employers who realize the value of an individual after he has been properly rehabilitated have granted the privilege of time away from work for the four-week course in many instances.

The monograph gives the design of the clinic, how it was constructed, and a description of the various rooms. Included are detailed plans of the sound-conditioned rooms which provide 90 decibels of attenuation. This was found necessary because of the noisy building in which the testing was done. Apparatus of various types is carefully described and although there has been some progress since the manual was written, the essential equipment which must be available in any audiology clinic is listed.

For someone who is planning a center where the hypoacousic and the dysphonic are assembled for rehabilitation, this manual offers an exceedingly fine description of a clinic which has functioned extremely well and which has been a model for many others in this country and abroad.

NORTON CANFIELD

PHYSIOLOGICAL FOUNDATIONS OF NEUROLOGY AND PSYCHIATRY. By Ernst Gellhorn. Minneapolis, The University of Minnesota Press, 1953. xiii + 556 pp. $8.50.

Dr. Gellhorn makes an heroic attempt to present and discuss his concepts of the physiological foundations of neurology and psychiatry, arrived at after a lifetime spent in the study of neurophysiology. His book is arranged

in six parts: (i) intrinsic and extrinsic factors regulating neuronal activity, (ii) contributions to the physiology and pathology of movements, (iii) the physiological basis of consciousness, (iv) some aspects of autonomic physiology, (v) integrations, and (vi) applications.

The first two sections could be better organized. His sentences are frequently cumbersome, as witness: "This survey has shown that the centrifugal transmission of motor discharges and the centripetal transport of afferent impulses are related in a simple manner to the excitatory processes set up in motor neurons and sense organs respectively." The outstanding chapters in these two sections are those on experimental convulsions and electromyography.

The section on the physiological basis of consciousness provides much better reading than the preceding sections. Here Dr. Gellhorn is handling a subject in which there are many as yet unexplored areas. It is implicit that the author realizes that the arguments presented in support of the hypotheses are tenuous at best. The section on some aspects of the autonomic physiology of the central nervous system where Dr. Gellhorn discusses neurohumeral and neuropharmacological aspects of the central nervous system is excellently done. In the fifth section Dr. Gellhorn discusses various integrative mechanisms, including the principles of neuroendocrine action, the physiological basis of emotion, factors involved in conditioning, homeostasis, and the constancy of the external environment. The last section, on applications, represents the synthesis and application of the information presented in the preceding sections of the book. Dr. Gellhorn's discussion of schizophrenia, shock and carbon dioxide therapy is stimulating and thought-provoking. This section is weakened by his omission of a presentation of some hypothesis to account for the effects of psychotherapeutic techniques consistent with the physiological principles applied to the therapy of psychoneuroses and functional psychoses.

The book is well indexed and contains an extensive bibliography of well over 1,200 references. It is not a text to be recommended to the average physician or medical student; rather it will be useful to physiologists, endocrinologists, internists, neurologists, neurosurgeons, and psychiatrists.

J. M. Q.

EXISTENTIAL PSYCHOANALYSIS. By Jean-Paul Sartre. New York, Philosophical Library, 1953. Translated with an introduction by Hazel E. Barnes of the University of Colorado. x + 275 pp. $4.75.

This book consists of two chapters from the author's *Being and Nothingness* (L'être et le néant), with the rest to be published later in a separate volume. It presents a theoretical basis for certain criticisms and revisions of Freudian concepts, in keeping with the existentialist orientation of Heidegger as modified by Sartre. It reinterprets such concepts as determinism, the unconscious, the topographic description of personality structure, the rôle of censorship and repression, etc. In relation to the concept of the unconscious, for example, Sartre "makes the psychic art coextensive with consciousness" (p. 76), but then points out that what is conscious is not neces-

sarily *known* to the subject; *"quite the contrary."* The central importance of *choice* is emphasized throughout: "Since what the method seeks is a *choice of being* at the same time as a *being*, it must reduce particular behavior patterns to fundamental relations—not of sexuality or of will to power, but of *being*—which are expressed in this behavior. . . . It will utilize the comprehension of being which characterizes the investigator inasmuch as he is himself a human reality; and as it seeks to detach being from its symbolic expressions, it will have to rediscover each time on the basis of a comparative study of acts and attitudes, a symbol destined to decipher them" (p. 88). "The behavior studied by this psychoanalysis will include not only dreams, failures, obsessions and neuroses, but also and especially the thoughts of waking life, successfully adjusted acts, style, etc. This psychoanalysis has not yet found its Freud" (p. 89).

Sartre discusses as particular illustrations of his point of view such psychological problems as desire, curiosity, play (there is an interesting existentialist analysis of the act and situations of skiing), generosity, how to give up smoking (p. 147), sliminess, and the significance of erogenous body zones. The final chapter, *Bad Faith*, is a discussion of the rôle of conscious choice. As put in the translator's introduction, "To be in bad faith means to be guilty of refusing to exist as a human being, and of pretending that one is a thing. A man in bad faith blames others or his environment for making him what he is instead of recognizing that he has made himself. He refuses to take responsibility for the self which by his acts he is creating."

Sartre's psychological descriptions are often vivid and illuminating, often possessing a literary rather than a psychodynamic flavor. They are full of flashes of insight, but lacking in the systematic view of personality function which is the hallmark of clinical training and study. On the other hand, the book presents serious challenge of the adequacy of nineteenth century scientific materialism in supporting the assumptions underlying the dynamic psychology of Freud. From this point of view, if from no other, the book deserves thoughtful study by the clinical psychoanalyst. Actually, Sartre accepts to a very considerable extent Freud's observations and conclusions; his interest is mainly in finding for them an appropriate place in his own philosophical framework.

JULES V. COLEMAN

THE CHILD, HIS PARENTS AND THE NURSE. By F. G. Blake. Philadelphia, J. B. Lippincott Co., 1954. xvii + 740 pp. $5.00.

To our knowledge this is the first textbook that attempts to provide the nurse with the specialized knowledge necessary for comprehensive child care. In many respects it reflects a new era in nursing education and practice, one in which the emphasis is not limited to technical procedures and knowledge of disease processes (disease-oriented care) but broadened to include a better understanding of the needs of children and parents (patient-oriented care) in health as well as disease.

Obviously written against a broad background of experience and careful observation, this book relates a vast amount of current knowledge about children to the practical problems with which nurses are concerned. It comprises, essentially, a discussion of the behavior of children and parents; the psychological, physical, physiological, and social growth of children; the interpersonal relationships which exist between child, parent, and nurse; the feelings and attitudes of children and parents toward illness and hospitalization; and the feelings and attitudes of the nurse in a number of situations. The text delineates the significant and unique contributions to mental health to be made by the nurse in her day-to-day activities. By helping her to new insights and a better understanding of children and parents, publications of this kind will increase the nurse's ability to *see*, to *hear*, and to *feel* what is going on around her, her understanding of what children are like at various ages, and her awareness of the questions and concerns which characterize each age period.

We believe that this book will make a real contribution to nursing education and child care in this country. It may be read and reread with profit by all those who work with children and parents.

MORRIS GREEN

WHO TECHNICAL REPORT. Series No. 66. Joint ILO/WHO Committee on Occupational Health. 2d Report. New York, Columbia University Press, 1953. 33 pp. Sw.fr. 0.80.

This 30-page booklet contains a substantial amount of information about modern occupational health principles and practices as they apply throughout the world. It starts from the premise that:

"Occupational health should aim at: the promotion and maintenance of the highest degree of physical, mental, and social well-being of workers in all occupations; the prevention among workers of departures from health caused by their working conditions; the protection of workers in their employment from risks resulting from factors adverse to health; the placing and maintenance of the worker in an occupational environment adapted to his physiological and psychological equipment and, to summarize: the adaptation of work to man and of each man to his job."

There is discussion of: (1) Measures of general health protection of workers in places of employment (utilizing the latter as an avenue of approach to general health problems). (2) Notification of occupational diseases. (3) Organization of comprehensive health-service programmes in large and small plants, and in agricultural enterprises. (4) Methods of cooperation between public health and industrial health services and of implementation of existing industrial health legislation and standards.

The report suggests substantial agreement among representatives of many countries as to basic considerations in occupational health. Thus the need is recognized of designing an occupational health service to meet the needs of the particular industry in the particular country where it operates. Specific services should be based on an intelligent evaluation, and if possible a survey, of the major health problems of the group of workers served. Al-

though occupational health services are usually offered to workers in the plant dispensary, the employer may find it desirable to supply complete medical care for a worker and his family if community health services are non-existent or grossly inadequate.

Nutrition, communicable disease control, measures for women and children, mental health, environmental sanitation, and health education are discussed under Section 1. In the section on mental health there is some suggestion that the health service may play a decisive operating rôle. This indicates lack of recognition by the committee that mental health as well as other objectives can be attained only with highly developed and progressive management teams. The contribution of organized labor is important also. However, good mental health depends on the management primarily, and the use of industrial psychologists in placement of workers (as suggested in the report) is probably far less important than the training of top management, supervisors, and foremen in handling and understanding workers.

In Section 2 on notification of occupational diseases, the practical nature of the report is evident. The committee modifies in a major way the conclusions of the International Labour Conference, particularly "the conclusion that the regulations are to include a minimum schedule of notifiable diseases and that this schedule is to be international in character." Pointing out (albeit most politely) that this purpose could lead only to international confusion, litigation, and unnecessary record keeping, the committee concludes "that the use of broad occupational designations (with expressly no limiting implication of causation) alongside particular diseases or poisonings would assist persons who have the duty of notifying, and the worker in identifying, occurrences which should be notified.

Section 3 includes under "Comprehensive programme in large plants," subsections on medical examinations; prevention, medical care and first-aid; supervision of working environment; health education and counselling; maintenance and analysis of records; premises; and personnel. The latter is excellent, noting about the physician what has been generally emphasized, that "his integrity must be unassailable." There is a good section on the functions of the nurse.

The section on services for agricultural enterprises points up the international character of this report by reference to a number of problems of predominantly agricultural countries with underdeveloped industries.

Section 4 on co-operation between public health and industrial health services is of interest, but seems a little shaky on the question whether or not occupational health is a part of public health. Occupational health is by definition a part of public health, operating as it does in relation to well-defined groups of the public. Until all interested labor organizations as well as health organizations recognize this, jurisdictional disputes will continue. It is to be hoped that there will be international recognition of the unity of public health by the time the next report is written.

The next to last paragraph of the bulletin is of more than passing interest, coming from ILO/WHO. "One approach which has been successful in some countries in improving the co-operation of official agencies among

themselves and with industry is worthy of consideration. Compulsion by meticulous enforcement of legislation may lead to reticence on the part of certain managements to provide welfare measures. This may also lead in certain instances to failure to cooperate with official agencies. An important approach is to stimulate the concept of promotion of industrial welfare and health, irrespective of legislation, by officially sanctioned incentives or rewards (such as insurance merit-rating, tax reduction, etc.) to those industrial establishments which provide welfare measures beyond the minimum prescribed standards."

In conclusion this report, though it scarcely reads like a novel, being full of "administrative" language, carries a message of encouragement to those who believe that occupational health services have the greatest available untapped opportunities for improvement of health of people everywhere.

J. WISTER MEIGS

ZUR GESCHICHTE DES MEDIZINISCHEN LEHRPLANS UND UNTERRICHTS DER UNIVERSITÄT FREIBURG I. BR. By E. Th. Nauck. Freiburg im Breisgau, Kommissionsverlag Eberhard Albert Universitätsbuchhandlung, 1952. 128 pp. DM 4.20.

This account of the history and development of medical education at the University of Freiburg opens with the reminder that until the beginning of the eighteenth century German medical teaching was largely based on classical works. Hippocrates, Galen, and the Arabic writers were still the chief authorities. Eighteenth century reforms began the release of medical science; it has since come a long way. The content and techniques of medical education have also progressed; the stages of their growth at Freiburg are carefully detailed in this *History*. Appended are summaries of the medical curriculum as it was described in the fifteenth century and later, a list of the medical faculty in 1460-1669 and of medical graduates in 1500-1599, an account of the introduction of the microscope as a teaching aid and of the development of histology, a plan for the reform of medical education in 1765-1767, and other fascinating records which are curiously reminiscent of the catalogue and administrative reports of a modern medical school.

Sources of information are carefully documented. A bibliography and indices are included. Printing and paper binding are satisfactory. The book will be of special value to historians but contains much to interest teachers of medicine and their students.

This *History* is the second volume of the *Beiträge zur Freiburger Wissenschafts- und Universitätsgeschichte.*

THOMAS R. FORBES

THE RELATION OF LEAN BODY WEIGHT TO METABOLISM AND SOME CONSEQUENT SYSTEMATIZATIONS. Albert R. Behnke, Ed. Ann. New York Acad. Sci., 1953. pp. 1095-1142. $1.25.

The author's studies of measurements and estimations of the mass and composition of the human body are generally recognized as contributions of

the first importance. Behnke has demonstrated that the "lean body mass," since it represents the active tissue of a person, is superior to reference standards hitherto proposed for the estimation of physiological functions such as the masses of various organs and tissues and the volumes of the different fluid compartments in the body. In the present article, which has been reprinted as a short paper-covered monograph, the same principle has been applied to the study of the relation of the lean body mass to oxygen consumption. A large part of the article is devoted to an ingenious mathematical comparison of lean body weight with other reference standards for basal metabolism; but there is in addition a discussion of the implications of this correlation. The paper, which was awarded an A. Cressy Morrison Prize in Natural Science in 1952 by the New York Academy of Sciences, deserves careful consideration by all who are interested in anthropometric measurements and metabolic standards. There is a useful bibliography of fifty-nine references.

JOHN P. PETERS

NUCLEAR PHYSICS. By W. Heisenberg. New York, Philosophical Library, 1953. ix + 225 pp. $4.75.

This small, non-mathematical, and very readable book on nuclear physics is from the pen of one of the most distinguished theoretical physicists of the day. Heisenberg originated many of the fundamental ideas of quantum mechanics, and for these discoveries he was awarded a Nobel prize. During the last war he directed some of the nuclear energy research in Germany. At present he is the director of the Max Planck Institute of Physics at Göttingen.

The first, a German, edition of this book, published during the war, was based on a series of lectures given to electrical engineers. This English edition is a translation of the latest revision in 1948. Since there is no coverage of the very extensive developments in nuclear physics in the past five years, this book is now to be recommended chiefly as an excellent coverage of the history and principles of nuclear physics. It should be of the greatest value to workers in other branches of science and technology who now find that they must have a greater knowledge of the radiations from atomic nuclei than they remember from their sophomore physics course. I guarantee that this semi-popular discussion of the nucleus is authoritative, and that there is practically not a single mathematical equation in the entire book.

W. W. WATSON

GENERAL VIROLOGY. By S. E. Luria. New York, John Wiley & Sons, Inc., 1953. xiii + 427 pp. $8.50.

One might say that Beijerinck's *contagium vivum fluidum* has at last come of age, for not only is Dr. Luria's book the first general virology text written entirely by one person, but the approach is one that results from the establishment of sufficient general principles to allow the subject to be attacked from the viewpoint of the viruses themselves rather than of the

diseases they produce. Thus the following topics are discussed equally for plant, animal, and bacterial viruses: morphology, chemical composition, serological reactions, environmental factors influencing the virus, virus-host interactions, growth of viruses, interference phenomena, variation of viruses, transmission, and ecological considerations. The subject is concluded with chapters that deal with viruses in relation to tumors and with the origin and nature of viruses. These last chapters are, for this reviewer at least, the most exciting portions of the book; the earlier sections form a firm basis for a more or less philosophical discussion of such controversial topics as the comparison of viruses and normal cytoplasmic constituents in their relationship to cells and the evolutionary processes involved in the development of this relationship. The presentation of the subject is excellent indeed.

It is fortunate that the very last chapter on Rickettsiae is entitled "Appendix," for it treats these organisms rather cursorily and without the excellent critical remarks that characterize the remainder of the text.

This is a book that was written for graduate students in virology, but there is not doubt that all who are interested in living processes, normal and pathological, would profit immensely by its perusal. The format is easy on the eyes. Typographical errors are few but seem to increase with increasing page numbers. There are 697 references; a good number of these are review articles with many additional bibliographies.

LIZBETH M. KRAFT

TRAUMATISMOS MEDULARES. By Victor Soriano. Buenos Aires, Argentina, Editorial Labor, 1953. 357 pp.

Some of the main symptoms of spinal cord injuries are discussed in this text as separate problems, presenting in a clear and attractive manner the physio-pathology, clinical description, and therapeutics of these symptoms. Some parts of the book are rather elementary but altogether it will be a very useful contribution to the Spanish reader.

The first chapter deals with etiology and gives a detailed description of civil life accidents with less emphasis on war injuries. Pathology, x-ray examination, first aid, and hospital care are the subjects of a brief but well-documented chapter. In presenting the problems of total and partial section of the spinal cord the experiences of the author add great interest to the material presented. Several case histories illustrate the different types of etiology, and the evolution and progress of symptoms. In concluding this section it is emphasized that disturbances of sensation rather than motility are of primary significance in determining prognosis.

Rehabilitation of the motor functions includes physical therapy and surgical techniques, which are presented wisely and conservatively. The chapter on bladder function gives a good anatomical and physiological background for better understanding and management of vesical difficulties; tidal drainage is described with special care. Short chapters are devoted to some aspects of the paraplegic, as sex, autonomic system, intestinal disturbances,

scars, metabolic disturbances, psychological care, and spinal cord regeneration.

The bibliography is generous and well selected. The book is warmly dedicated to Professor John F. Fulton under whose direction the author conducted an interesting research project at Yale University School of Medicine during the year 1946-1947. The collaboration of the author's wife, who executed most of the excellent drawings which illustrate the book, is also gratefully acknowledged.

JOSÉ M. R. DELGADO

THE QUANTITATION OF MIXTURES OF HEMOGLOBIN DERIVATIVES BY PHOTOELECTRIC SPECTROPHOTOMETRY. By Francis T. Hunter. Springfield, Ill., Charles C Thomas, 1951. xx + 226 pp., 8 figs., 49 charts. $8.50.

This book is based on long experience in the quantitative determination of hemoglobin and its derivatives in small blood samples. The spectrophotometry of mixed pigments requires the use of a narrow spectral band, as given by an instrument of the prism or diffraction-grating type. Filter photometers (photoelectric colorimeters) are dismissed with the statement that their conversion charts are usually curves and not straight lines. After developing the algebraic relations between optical density and concentrations of two and three components, the author gives a detailed treatment of the construction of charts and nomograms.

Methods are described for the determination of oxyhemoglobin, hemochromogen, carbon monoxide saturation, oxygen saturation, neutral methomoglobin, hemoglobin pigments in serum, and erythrocyte volume. The concentration of a pigment is expressed as the oxygen capacity of a solution containing an equivalent amount of hemoglobin, and all calibrations are based on manometric Van Slyke determinations of oxygen capacity. The oxygen capacity of one gram of hemoglobin is taken as 1.38 ml., but no evidence in support of this figure is cited. The oxygen capacity of diluted blood is expressed in volumes per liter, which seems to mean milliliters per liter.

The methods are clearly described and appear to be well adapted to routine laboratory use. The mathematical chapters should be useful not only to analysts of blood, but also to those concerned with other light-absorbing mixtures.

DAVID I. HITCHCOCK

TRAITMENT DES HÉMORRAGIES. By J.-P. Soulier. Paris, Éditions Médicales Flammarion, 1953. 217 pp. 1.400 francs.

This is a practical guide by an active specialist, who is "Medicin-Assistant des Hôpitaux de Paris" and "Chef de Laboratoire au Centre National de Transfusion Sanguine et de Recherches Hématotogiques." The author's critical appraisal is often brief and eloquent. He disposes of blood coagulation theory in two short paragraphs (p. 8) aptly entitled, "Rappel des Notions Actuelles. . . ." In rejecting a dubious therapeutic measure, he

comments (p. 82), "Après de publications initiales optimistes, ont succédé des rapports négatifs."

In the introduction, it becomes clear that French laboratory methods are much like ours. The Lee-White method and the Quick test are standard procedures. On the other hand, the Continental workers seem to place more emphasis than we do on the *in vitro* heparin tolerance. Later portions of the book refer to interesting and ingenious applications of the heparin tolerance test, as used by Soulier and Le Bolloc'h. Page 65 discusses the paradoxical hemorrhages encountered during dicumarol therapy and emphasizes what Dr. Le Bolloc'h mentioned when she visited Yale—that their heparin tolerance test is believed to be a safer guide to dicumarol therapy than is the Quick test.

The first main part of the book is devoted to therapeutic agents. Soulier has reservations concerning the effectiveness of adrenochrome as a hemostatic drug. For the counteraction of heparin, he prefers protamine sulfate to toluidine blue. Both drugs can cause undesirable reactions, which Soulier discusses. For local hemostasis, he prefers gelatin foam to oxidized cellulose. For hemophilia he prefers dry plasma, which is prepared from fresh blood at the Centre Nationale de Transfusion. The patient can keep this material at home, or take it with him on a trip. This advance in medical management illustrates one of the many advantages of centralized blood banking.

Soulier does not consider fraction I to represent a real advance over dry plasma in the treatment of hemophilia. For acute fibrinogenopenia he recommends fresh whole blood in quantities as high as two or three liters; but as appears later in the book (p. 151), this is not always successful.

The second part of the book describes the diagnosis and treatment of the hemorrhagic syndromes. The chapters on hemophilia, the hypoprothrombinemias, and the thrombopenic purpuras are especially detailed and explicit. It is of interest that Soulier's experience with about eight hundred cases of hemorrhagic disorder includes 29 cases of Willebrand's thrombopathy (normal platelet count and clot retraction, with prolonged bleeding time), but not a single case of Glanzmann's thrombasthenia (isolated defect in clot retraction).

Several specialists collaborated in the third part of the book(which deals with hemorrhages according to site: urinary tract, genital tract (one chapter on gynecological hemorrhages, two on obstetrical hemorrhages), nervous system, eye, ear, nose and throat, mouth, respiratory tract, digestive tract, and skin (dermatologic purpuras).

J. H. MILSTONE

INFRARED ABSORPTION SPECTRA OF STEROIDS (AN ATLAS). By Konrad Dobriner, E. R. Katzenellenbogen, and R. Norman Jones. New York, Interscience Publishers, Inc., 1953. xiv + 308 pp. $11.50.

One of the most important advances in the study of the biochemistry of steroids has been the introduction of infrared analysis. Early studies by Drs. Furchgott, Rosenkrantz, and Shorr at Cornell University Medical College

demonstrated the importance of this technic for the identification of steroids. This Atlas represents the important contributions to this field made by the Sloan-Kettering Institute Group, particularly the late Dr. Konrad Dobriner, and Dr. R. Norman Jones originally of Queen's University and now at laboratories of the National Research Council of Canada. As Dr. C. P. Rhoads points out in the introduction, the success of the infrared project which resulted in part in the issue of this Atlas was in part due to cooperation of investigators at the American Cyanamid Company, particularly Dr. R. B. Barnes, as well as Dr. James Hardy of Cornell University Medical College.

The publication of this Atlas now places in the hands of all interested investigators the infrared curves of some 306 compounds including a great number of steroids of physiological importance. The bulk of the spectra are of steroid hormones and their metabolites but representative curves of bile acids, steroid sapogenins, cardiac aglycones, and steroid alkaloids are included. The curves are plotted in terms of wave number units and percentage absorption. These plots cannot be superimposed upon transmission recording obtained in individual laboratories. The volume contains short sections on experimental technic and the use of spectra in structural determinations. This latter section might have been profitably expanded.

This carefully prepared volume is a valuable contribution to the steroid literature and I am sure copies will be found in all laboratories where studies on the biochemistry of steroids are being done.

RALPH I. DORFMAN

GLOBAL EPIDEMIOLOGY—A GEOGRAPHY OF DISEASE AND SANITATION. Volume Three, THE NEAR AND MIDDLE EAST. By J. B. Simmons, T. F. Whayne, G. W. Anderson, H. M. Horack, R. A. Thomas, and Collaborators. Philadelphia, J. B. Lippincott Co., 1954. xxiv + 357 pp., 18 maps. $12.00.

Encyclopedias are of increasing value as they are brought to completion, and the appearance of the third volume in this systematic digest of world health and hygienic conditions is cause for congratulation to the authors and of satisfaction to the interested public.

This public will prove much larger than the medical and allied professions, for the volume should be valuable to all whose interests in any way are involved in this area—in the organization of transportation to and within the region, those considering investments or other business ventures, anthropologists, sociologists, missionaries, travelers—and to officials of the State, Armed Forces, and Commerce Departments.

This, perhaps prejudiced, reader found the volume fascinating. Health conditions which resemble those of the Middle Ages still persist in the Near East. In this year of grace, there are countries where the mortality in infants before the age of five is estimated at 500 per 1,000 live births, where annual smallpox epidemics occur, where there is no sewerage system for a country of 170,000 square miles and five million inhabitants, where the ratio of doctors to the population varies from about 1:2,000 in the best

areas to 1:20,000 in rural regions. Equally striking are the advances in certain areas which have been influenced by Western thought on the value of health and hygiene and the improvements in even the most unhealthy countries when modern control methods have been used in restricted regions, *e.g.* in the control of malaria. To what extent matters will change, if any, will depend in part on the intellectual orientation and political future of this area, perhaps as it will develop in our lifetime. Even if involuntary, our interest is committed as taxpayers and as participants in a government itself with world-wide commitments.

The extraordinary scope of this work was made possible by drawing upon the material accumulated by the Preventive Medicine Service of the United States Army, in the functioning of which several of the authors have played prominent parts. To review what is essentially a skillfully compiled and critically edited series of abstracts is perhaps best done by indicating the contents. Each of the seventeen chapters has the following five subdivisions: (1) Geography and Climate, (2) Population, (including anthropological data of interest), Vital Statistics, Social Economy, Food and Nutrition and Housing, (3) Water Supplies, Waste disposal, Flora and Fauna, Food Sanitation, (4) Health Organizations, Medical Institutions and Personnel, (5) Communicable diseases classified by means of spread, and nutritional diseases, and miscellaneous conditions. Each chapter is terminated with a summary and bibliography. The seventeen countries treated are: Cyprus, Iraq, Israel, Jordan, Lebanon, Syria, Afghanistan, Iran, Turkey, Aden, Bahrain, Kuwait, Muscat and Oman, Qatar, Saudi Arabia, Trucial Oman, and Yemen. There is a concluding and valuable appendix "Health Hints for the Tropics," containing such useful data as immunization requirements (for all countries), routine immunization procedures, and dosages for children of the immunizing agents, as well as recommendations for non-medical persons concerning the protection of food and drink, methods of prophylaxis of malaria, and other items.

This is a most serviceable book and it is difficult to see how it could be improved within the allotted space. It is to be anticipated that the authors will complete the series.

DAVID WEINMAN

MR. TOMPKINS LEARNS THE FACTS OF LIFE. By George Gamow. New York, Cambridge University Press, 1953. xii + 88 pp. $2.75.

"Inside Mr. Tompkins" might have been another title for this new book in the Mr. T. series. It recounts three trips taken by the explorer around his own body which has become microscopic in size by devious ways and means.

In Trip No. 1 Mr. T. manages to get injected into his own bloodstream. He sightsees about in the circulation, "hitching a ride" on a red cell in the company of a Popeye-faced Vitamin C molecule and several apple-sized flu viruses. The jaunt ends in the digestive tract, where Mr. T. signs out against advice into the lumen and wakes up seconds before getting digested (in his own juices).

Trip No. 2 takes our tourist inside the cell whence he contemplates chromosomes, genes, and other templates moulding his destiny. Guiding him along the paths of phylo- and ontogeny is a certain Mr. Netherlander of questionable descent.

Trip No. 3 is begun by courtesy of "Maniac," the conceited electronic computer, who, amongst other accomplishments, is well versed in the King's English. Thus he is an able explainer of analogies, especially relative to the primitive, non-electronic predecessor of the brain Mr. T. happens to have. The revelations culminate when our explorer meets in the forest of his Betz cells a gentle Russian and his less gentle, but well-reflexed dogs, and under their guidance learns much of synapses and dreams.

Thus Mr. T. gets to know of the workings of his body, and in a final trip—this time to an "outside" lecture—learns how the details might be fitted into the world around us.

A delightful little volume, for the smooth and painless induction of biological interest.

N. A. H.

DE ANEURYSMATIBUS. THE LATIN TEXT OF ROME, 1745. By Giovanni Maria Lancisi. Revised with translation and notes by Wilmer Cave Wright. New York, The Macmillian Company, 1952. xxxv + 362 pp. $7.50.

Lancisi, whose major works were published in the first half of the eighteenth century, is known for several things, as for instance his advocacy of cinchona bark in the treatment of miasmal fever. However, for thoroughness and sheer brilliance, his greatest monument is almost certainly this treatise on the aneurysms of the heart and blood vessels. There are indeed some errors of concept. The theory that erosion of the outside of arteries by acrid lymph produced syphilitic aneurysms, or his thinking, in the case of a young man exhibiting an aneurysm of the popliteal artery, that its nature was venereal because of the lustful habits of the victim, are examples of probable error. These constitute but trivial flaws in a masterpiece impressive for its precision of detail and particularly for its logical development.

Although he despised the empiricists and was, by nature, a strong believer in general principles, these same principles derived in the first place from his keen powers of observation and understanding. The simplicity of his methods is refreshing for, ever since the time of Claude Bernard physicians have come to believe more and more that medical science must be chiefly experimental and necessarily complex. In the search for the clever theory and the ingenious test thereof, the art of simple clinical observation, as practised earlier by Lancisi, frequently has been overlooked. Lancisi demonstrated that without any laboratory aids except the dissecting scalpel, without, in fact, looking farther than naturally occurring phenomena for his "experiments," the perceptive physician could contribute firm and useful information to the total fund of medical knowledge.

Aneurysms are classified as, on the one hand, true, including those due to trauma, contusion, and weakness of vessel walls (e.g., syphilis), and, on the other hand, spurious, being those due to increased impetus of blood

beating against the vessel walls. Lancisi includes dilatation and hypertrophy of the heart in his work, and here some of his comments have the same sophistication that one might expect of a modern pathologist describing the detailed post-mortem findings of a case of heart failure.

The history of medicine is a complex discipline, but one of its most fascinating aspects is discovering for oneself in the record of the past a first-rate mind using well-developed natural senses. Therein lies the reward for the reader of Lancisi's work on aneurysms.

The translation and annotations of Mrs. Wright are excellent.

FRANK D. GRAY, JR.

ABRAHAM TREMBLEY OF GENEVA; SCIENTIST AND PHILOSOPHER, 1710-1784. By John R. Baker. London, Edward Arnold & Co., 1952. xix + 259 pp., 52 illustrations. $7.50.

This book retrieves from obscurity a biologist who achieved important observations and discoveries. He described with precision the reproduction of the polyp *Hydra* and of related primitive organisms. He learned that from their fragments can be regenerated complete bodies—multiplication by division. He proved asexual reproduction of polyps by budding. He first observed cell division. He successfully and permanently grafted animal tissues. He was an artist, a skilled teacher, an interesting friend. His description of the regeneration of polyps, it may be added, caused a major stir in the Académie Royale des Sciences and the Royal Society, won him the latter's Copley Medal, and attracted the literary barbs of Smollett, Fielding, Goldsmith, and Voltaire.

Professor Baker, a distinguished cytologist, writes with thoroughness and respect for detail. Statements are carefully documented. An excellent series of drawings and photographs has been assembled by the author. The book is interesting, clear, and well organized. A bibliography and index are appended.

THOMAS R. FORBES

MICHAEL SERVETUS. A translation of his geographical, medical and astrological writings with introductions and notes. By Charles Donald O'Malley. Philadelphia, American Philosophical Society, 1953. 208 pp., 7 plates. $3.00.

"Controversial" is really too mild a characterization of Miguel Serveto. Professor O'Malley's phrase, "child of calamity," is more suitable. Possessor of a vigorous and original mind, the great Spanish scholar distinguished himself as physician, theologian, and theorist. Yet by his irritant heresies and magnificent indiscretion, to say nothing of a measure of dogmatism and reformer's zeal, Servetus incurred the banning of one of his books and the burning by the authorities of his most famous work (the *Christianismi restitutio,* in which occurs the remarkable description of the pulmonary circulation, here presented as one of Professor O'Malley's translations.) He was obliged to adopt a pseudonym, not as a whim but for

his own safety, eventually dared live neither in France, Switzerland, Germany, nor Spain, and perished at the stake because he had antagonized the church in generaland Calvin in particular.

Much of Servetus' story is told in the compact and absorbing introductions with which Dr. O'Malley sets the scene for each of his excellent translations of these "non-theological writings." In the latter, too, there is much interesting reading—Galenic dogma, a display of ingredients for medicinal syrups, a picture of a brisk and caustic medical controversy, a defense of astrology.

Dr. O'Malley should be congratulated as well for his inclusion of facsimile reproductions of the title pages of the usually inaccessible original publications. Excellent printing adds to the reader's enjoyment of this book. The latter is Volume 34 of the Memoirs of the American Philosophical Society and Publication No. 32 in the Monograph Series of the Historical Library, Yale University School of Medicine.

THOMAS R. FORBES

for a KNOWN response

Fully evaluated clinically

...and known as an agent of choice in the treatment of infections caused by gram-positive and gram-negative pathogens, spirochetes, rickettsiae, certain large viruses and protozoa.

True broad-spectrum action

"Terramycin appears to have [a] most widespread effective range...."[1]

Rapidly absorbed

"Terramycin administered orally was absorbed rapidly with the appearance of antibacterial activity in the serum within 1 hour of administration."[2]

Promptly effective

Many "patients...improved rapidly"[3] with return of temperature to normal often noted within twenty-four hours.

Well tolerated

"Terramycin is generally well tolerated...."[4]

Terramycin®

Brand of oxytetracycline

1 Cowart, E. E., Jr.: Mississippi Doctor 29:278 (April) 1952
2 Sayer, R. J., et al.: Am. J. M. Sc. 221:256 (March) 1951
3 Knight, V.: New York State J. Med. 50:2173 (Sept. 15) 1950
4 Trafton, H. M., and Libd, H. E.: J. Urol. 69:316 (Feb.) 1953

Pfizer

PFIZER LABORATORIES, Brooklyn 6, N. Y.
Division, Chas. Pfizer & Co., Inc.

announcing the ninth annual

SCHERING AWARD

for medical students

1954

$500 – First Place Award

$250 – Second Place Award

for the best papers on each of the following subjects

1. Modern Treatment of Infections and Allergic Disorders of the Eye
2. Prophylactic and Therapeutic Uses of Parenteral Antihistamines
3. Androgen Therapy in the Female

Closing Date: entry forms—before July 1, 1954
completed MSS—September 30, 1954

Information and Entry Forms: contact Dean's office or write

Schering CORPORATION · BLOOMFIELD, NEW JERSEY